JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXX WASHINGTON, D. C., FEBRUARY 1, 1925 NO. 3

ANATOMY OF THE VEGETATIVE ORGANS OF SUGAR CANE [1]

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INTRODUCTION

The vegetative organs of the grasses have been the subject of very extensive studies, and certain structures, like the vascular bundles of the stem, are familiar to elementary students of botany. The more difficult features of the inner structure of this group of plants have also received the close attention of plant anatomists, but it was only after many unsatisfactory attempts that a correct understanding was reached, as, for example, in the account by von Mohl (*13*)[2] of the relationship of the leaftraces. The peculiar and variable structure of the leaves was made the object of extensive studies especially by Duval-Jouve (*5*). Strasburger (*16*) has given an excellent account of the course of the leaf traces in *Zea mays*, a plant which in many respects is closely akin in structure to the sugar cane. The works of Kobus (*10*), Beneke (*1*), Wiesner (*19*), Krüger (*11*), Geerts (*6*), Dickhoff (*4*), Kuyper (*12*), Bremekamp (*2*, *3*), Kamerling (*8*), Venkatraman (*18*), and Takenuchi (*17*) deal with certain phases of the anatomy of sugar cane (*Saccharum officinarum* L.), and fragmentary accounts are found here and there in connection with studies of sugar-cane diseases.

Additional work on the anatomy of sugar cane has seemed desirable, since none of the existing publications give a complete and sufficiently detailed picture of the inner structure of the plant, and even these publications are accessible to but few.

THE PLANT

The variety Louisiana Purple (Black Cheribon) (pl. 1) which has been selected for study is a representative of the large group of varieties within the species *Saccharum officinarum*, or the so-called "noble canes" as distinguished from the more slender-stalked prolific-stooling groups of varieties, of which the well-known Uba is typical, and from the well-defined group of exceedingly thin-stemmed canes of northern India, like the Chunnee and Ruckee. The two latter groups of varieties exhibit differences which probably entitle them to specific rank, according to J. Jeswiet of the Proefstation voor de Java-Suikerindustrie.[3] The Louisiana Purple variety is a stout grass with solid juicy stems, broad flat leaves and large plumelike inflorescences.

The stem is from 3 to 5 m. tall and about 4 cm. thick; it is composed of nodes and internodes. The latter are commonly barrel-shaped or cylindrical and disposed linearly one above the other. They are of even thickness except in the underground part, where they taper to a cone (fig. 1).

[1] Received for publication May 2, 1924; issued April, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 221.

[3] Not published but communicated verbally by Jeswiet to E. W. Brandes, pathologist in charge, Office of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Dept. of Agriculture.

EXPLANATORY LEGEND FOR PLATE 1

Anatomy of the Vegetative Organs of Sugar Cane.

A.—Node and internode of mature stem of Louisiana Purple. The colors are natural except in the cut surface; the latter is a reproduction of the colors obtained by leaving the material in potassium bi-chromate. The vascular bundles appear white.

B.—Base of leafsheath. The sheath-joint appears as a light colored and smooth, somewhat protruding shoulder.

C.—Blade joint; the flanges of the joint are brown and pubescent.

D.—Cross section through a mature leaf. The chloroplasts are colored green; the lignified tissue red. ×234.

The internodes tend to increase in length acropetally, but in the apical region they become shorter again. The nodes are commonly thinner than the lnternodes. Above them, protected by the sheath, is the Keimring, which bears a bud and several rows of root primordia. All parts of the stem, with the exception of the Keimring, are covered with wax. In old stems these wax deposits peel off in places, thereby

The leaves are two-ranked and parallel-veined. Each leaf consists of two parts—the sheath and the blade. The sheath envelopes the stem with the margins overlapping, and as a result of this arrangement one leaf will form a right, the next in succession a left spiral. The sheath is widest at the base and gradually narrows, reaching its smallest diameter where it merges into the blade. The base of the sheath is swol-

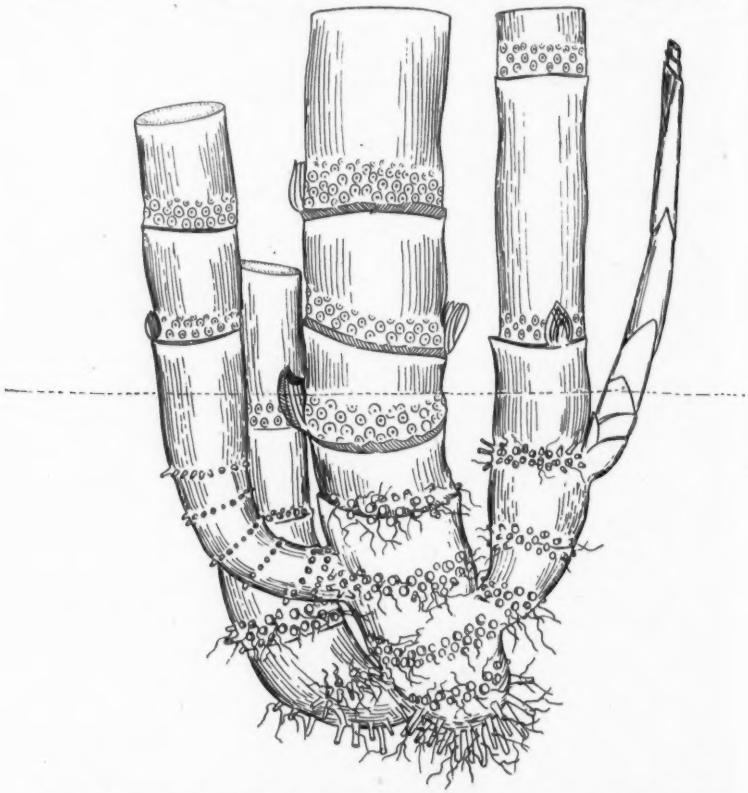


FIG. 1.—Diagrammatic drawing of part of stool of large sugar cane. New secondary and tertiary stems arise from the buds of the underground stem

often giving the surface of the cane a peculiar striation.

The buds in this variety appear as appressed conchate structures of varying form. They are slightly sunken in the stem, as indicated by a longitudinal groove, which only gradually loses itself in the upper part of the internode. In the lower, more mature parts of the stem the buds become more prominent, and the tips bend away from the axis instead of lying next to it.

len, forming a pseudojoint. At the junction of sheath and blade a similar joint is formed. On the inside of this blade joint there is a membranous, hyaline appendage, the ligule. The sheath has an average length of about 34 cm. and a width which is directly correlated with the thickness of the stem. In a given region the sheath is thickest at the middle, becoming gradually thinner toward the margins. The surface of the sheath is covered with hairs of

various types. These are most numerous at the two joints and along the median axis. At the base of the ligule, but arising from the inner epidermis of the sheath, are long silky cilia.

The blade is linear lanceolate, up to two meters long, and at the middle is from 5 to 7 cm. wide. There is a prominent midrib which projects from the lower surface and forms a groove in the upper. The leaf margin is denticulate with fine silicified teeth. The surface varies from hispid to pubescent with the longer, softer types of hair occurring on the lower surface.

The color of the cane is characteristic of the variety. Gradations, however, are induced by light and the age of the organ. The sheath is of a uniform green, which becomes pale toward the margins. Sometimes the basal part of the young organ shows a different color, a reddish or purplish tinge, which, however, disappears as the plant matures. The upper surface of the blade is a bright green, while the lower tends to take on a grayish tinge owing to the presence of hairs.

The root system of the cane, like that of other grasses, is composed of numerous fibrous roots. A distinct taproot is found only in seedlings and even here it is poorly developed, ceasing to grow after a brief period. The secondary lateral rootlets are much thinner than the primary ones and have but an ephemeral existence. When they become detached from the larger roots they leave abscission zones which may serve as infection courts for numerous parasites which, as is well known, invade the root system of the cane more readily than that of other plants.

The inflorescence forms a large open panicle. The flowers are arranged in small spikelets, each surrounded at the base by a tuft of silky hairs from two to three times the length of the spikelets. The latter are grouped in pairs: one sessile, the other pedicelled. Both spikelets are perfect and awnless. Each flower is subtended by two bracts which form the outer and the inner glumes. The outer glume is membranaceous, pointed, entire-margined and two-nerved. The inner glume is similar, but possesses a median keel. Continuing the two-ranked arrangement of the two glumes is the sterile lemma which is lanceolate, pointed, and practically without veins. The fertile lemma and palet of the typical grass flower are wanting. At the base of the flower, just inside the inner glume, are two thick, hyaline lodicules. These two lodicules and a small scale just inside of and inclosed by the sterile lemma may be considered the trimerous perianth of the flower. Lastly, the axis bears a whorl of three stamens and the ovary. The stamens have two-celled, versatile anthers, each suspended by a delicate filament. The ovary is short-stalked, erect, one-celled, and contains one ascending anatropous ovule. Owing to infertile or degenerate pollen in the Louisiana Purple variety, viable seeds are born only when foreign pollen is introduced.

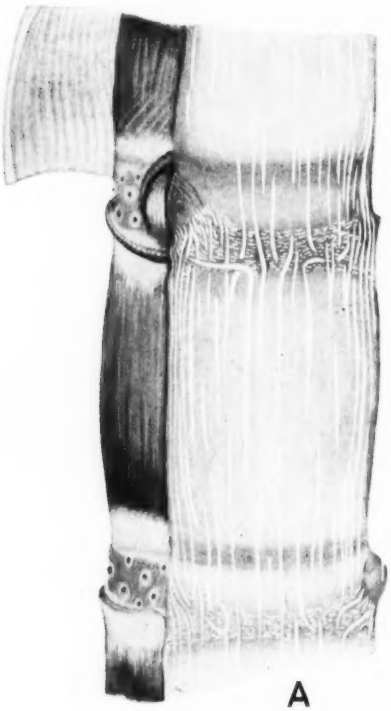
The fruit is small, about 1½ mm. long with a distinct constriction in the region opposite the embryo. It is indehiscent and included in the glumes and the sterile lemma. The pericarp is thin and intimately united with the testa. The comparatively large embryo lies in contact with the starchy endosperm by means of the cotyledon, which acts as an absorbing organ.

The sugar cane grows acropetally by a cone-shaped growing point. The increase in length of the stem, however, is due to an intercalary meristem at the base of each internode. The leaves are cut off by the apical growing point in rapid succession, and as new leaves appear a bud is formed in the axils of the older ones. The leaves grow much more rapidly than the stem, as can easily be seen in secondary shoots which have leaves several feet long, while the growing point is still underground. The formation of successive leaves and the subsequent growth in the intercalary zone divides the stem into segments, each consisting of a node and an internode. After the plant has reached a certain size, the lower underground buds initiate development and form secondary shoots, the amplification of this process resulting in stools which may contain scores or even hundreds of stalks.

ANATOMY

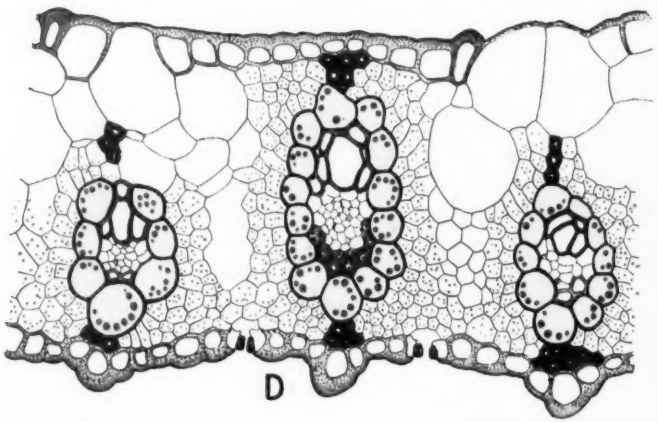
The material for study was grown in greenhouses at the Arlington Experiment Farm, Rosslyn, Va. The greenhouses had been especially constructed for the growing of sugar cane, in order to insure the most natural development of the plants.

The material taken from the greenhouse was studied while fresh and many of the photographs were obtained from free-hand sections of fresh material. For the purpose of securing permanent records, representative material was killed in Flemming's stronger solution, embedded, some in paraffin, some in celloidin, sectioned, and stained in the usual manner.


A

B

C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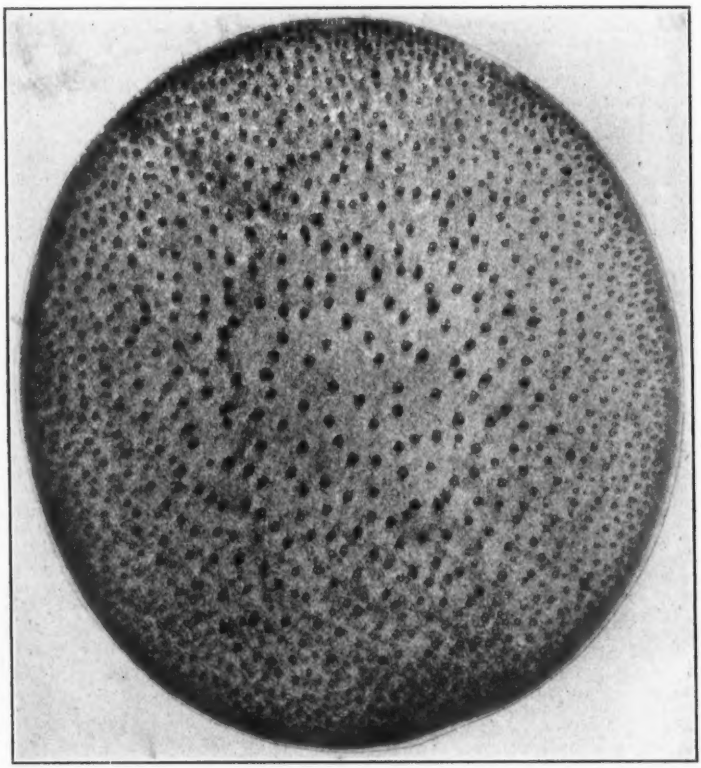


Cross section of a mature internode (1,275 vascular bundles). × 3

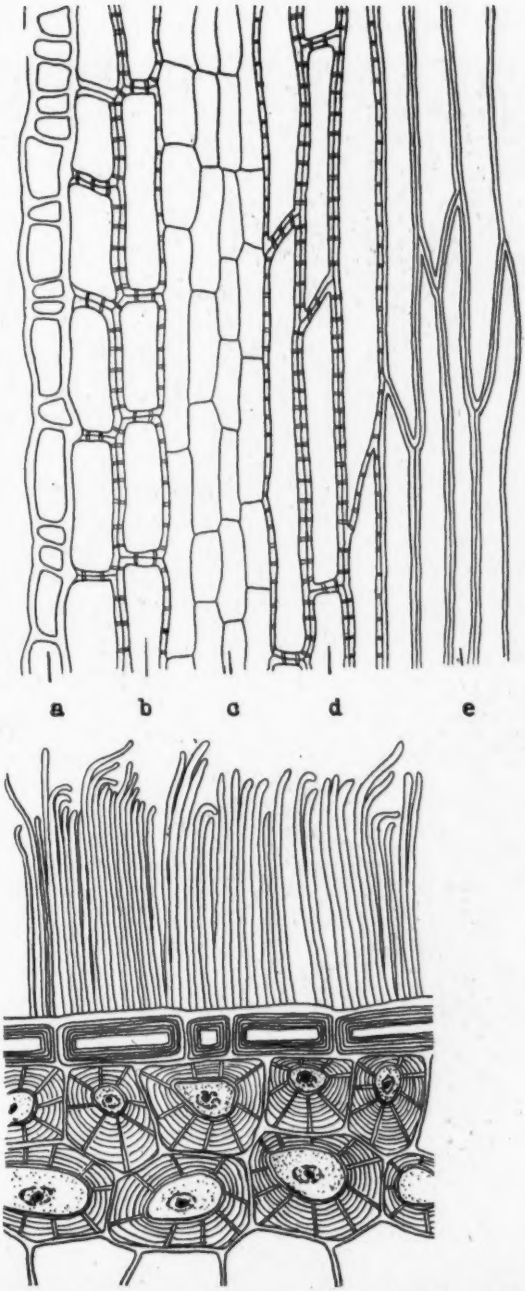
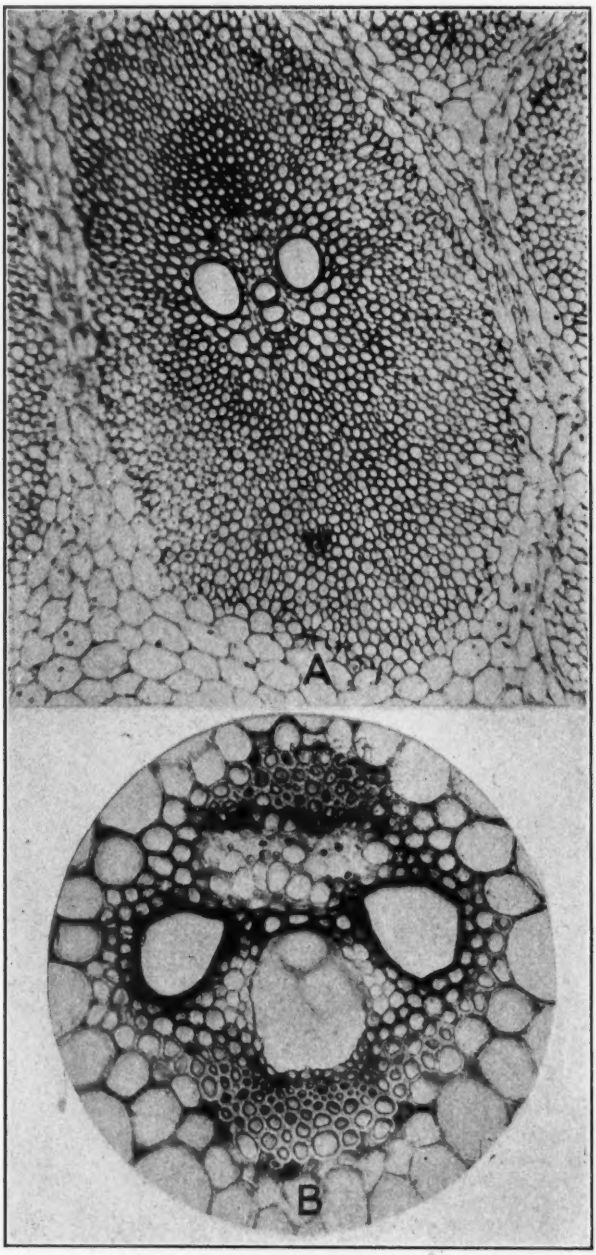


FIG. 2.—Longitudinal section through peripheral region of mature internode. × 360. *a*, Epidermis; *b* sclerenchymatous hypodermis; *c*, thin-walled cortical tissue; *d*, sclerenchymatous cortical tissue; *e*, sheath cells of peripheral stem bundles. Cross section through the nodal region of a mature stem. The epidermis is covered by a wax deposit in the form of densely crowded rods. The cells of the strongly lignified hypodermis have a large nucleus and cytoplasm. × 505.



A.—Large peripheral stem bundle. × 113
B.—Large bundle from the central region of the stem × 113

THE STEM

A node and an internode taken from the upper third of a large cane was used as representative material for the anatomical study of the stem. The internode was uniform, about 3½ cm. thick and 15 cm. long; the node was slightly thinner and exhibited regions of internal and external structural differences and will, therefore, in this discussion be subdivided into: (*a*) The node proper, which is limited above by the insertion of the leaf sheath; (*b*) the Keimring, which contains a bud and several rows of root primordia; (*c*) the intercalary meristem from which elongation of the internode takes place. These regions differ from each other, but because of the transition zones, however narrow, they will be treated in sequence rather than as altogether different entities.

A cross section of the internode (pl. 2) shows numerous vascular bundles embedded in parenchymatous tissue. Externally it is limited by a thick epidermis which, for further protection, is covered by a layer of wax. The vascular bundles are not arranged in a simple ring, but lie scattered throughout the section. Their number increases from the center toward the periphery, whereas their size gradually decreases. At the periphery the bundles are so small and so close together that they form practically a solid ring. The vascular tissue is separated from the epidermis by a cortex which varies in width and composition with different regions of the stem.

The parenchyma constitutes the filler between the bundles, except in the peripheral region, where it forms an uninterrupted layer comparable to the cortex of dicotyledonous plants. The parenchyma cells are thin-walled and separated by small intercellular spaces. In cross section the cells are roundish or somewhat elliptical, whereas vertically they have the form of short cylinders with straight or sometimes rounded ends. The depth of the cells varies with their position in the stem, those in the internode being elongated near the vascular bundles, but in the nodes the cells are very short regardless of their radial position.

The cortex forms a narrow band of tissue except in the Keimring where its width more than doubles. The two to four rows of cells next to the epidermis are small, thick-walled and lignified (pl. 15, A, and fig. 2). The vertical continuity of this sclerenchymatous mantel, however, is broken by the occasional interpolation of parenchymatous cells which abut externally on a stomate of the epidermis. This layer is followed by several rows of thin-walled parenchyma, the cells of which remain cellulose even in old and woody stems. There is a gradual transition from the cells of the cortex to those of the bundle parenchyma. The cells of this transition zone, which form the filler between the peripheral bundles, grow progressively larger centripetally and the walls become lignified wholly or in part. In the region of the Keimring the cortex is composed of uniformly small cells which remain cellulose. In very old stems, however, the two hypodermal layers may show partial lignification.

EPIDERMIS OF THE STEM.—An epidermis typically forms a single layer of cells, possesses stomates and produces outgrowths in the form of hairs. In the stem of the sugar cane, however, while stomates are sparingly developed, hairs are entirely wanting, though in a different variety they have been reported once by Soltwedel (*11, p. 28*).

The individual cells of the epidermis vary in size and form; on the whole, however, they are remarkably uniform. There are present two distinct types of cells alternating with one another; elongated rectangular cells with undulating walls, and short cells which occur singly or in pairs (pl. 5, C).

The long cells form four-sided prisms with a mean radial diameter of 9.8μ and a length varying between 54 and 184μ. The outer walls are greatly thickened and cuticularized. There is also present, especially in the region below the insertion of the leaf sheath, a wax deposit composed of a layer of densely crowded wax particles in the form of hooked rods (fig. 2). The walls of the epidermis are pierced by numerous pits, which are easily seen when the section is treated with chloral hydrate or some suitable clearing agent. In the region of the intercalary meristem the epidermal cells are very broad, and the walls less tortuous and comparatively thin (pl. 5, A).

The short cells are transversely rectangular, but the two components of the pair are rarely uniform. One is usually smaller, narrower tangentially and has silicified walls. The larger of the two has a tendency to be irregular; it is frequently pointed, which gives it the appearance of a wedge. Occasionally one or even both of the short cells are wanting, which results in the latter case in a continuous longitudinal row of long cells.

CENTRAL STEM BUNDLES.—In order to fully appreciate the different aspects

of the vascular bundles in the various regions of the stem, it seems best to take typical bundles, study their structure and note the changes that take place as these bundles pass through the nodal and internodal region of the stem.

The typical bundle, as found in the larger part of the cane, except in the peripheral region, is rhomboid in cross section. It is surrounded by a well-marked sclerenchymatous sheath which is most strongly developed on the inside and the outside of the bundle where it forms typical bundle caps (fig. 3). The vascular tissue of the bundle has xylem and phloem disposed collaterally in relation to each other. The protoxylem consists of annular and spiral elements. Below it is the protoxylem lacuna, a lysigenous cavity into which often project the remains of the first-formed annular vessels (pl. 3, B). Above and lateral to the protoxylem are two large vessels with medium long articulations which communicate by a single large pore. The vessels are surrounded by tangentially flattened parenchyma cells (pl. 4, A), which commonly possess reticulate thickenings. There is profuse pitting between the vessels and the parenchyma, but where a vessel directly adjoins the sheath

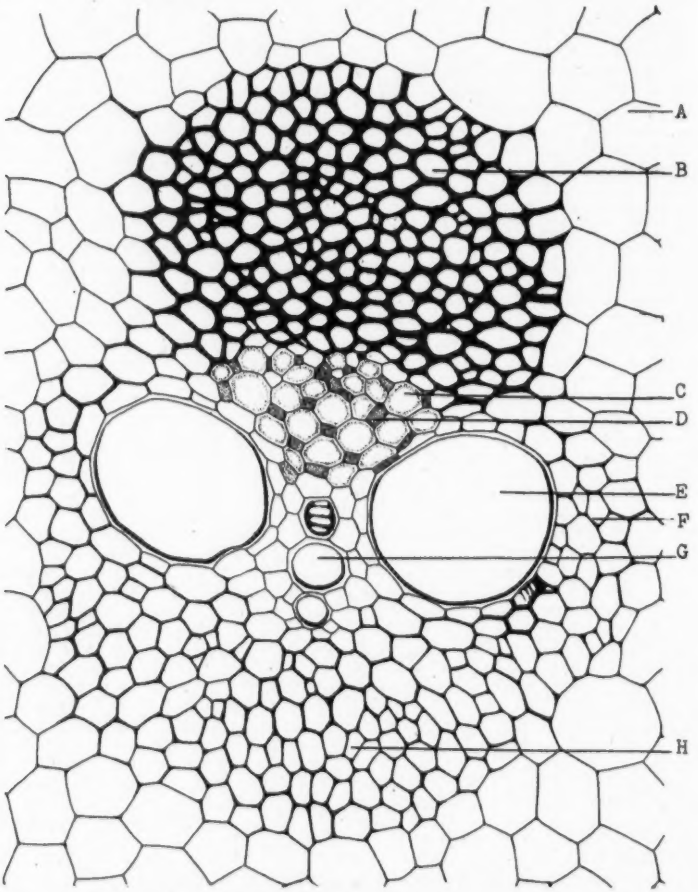
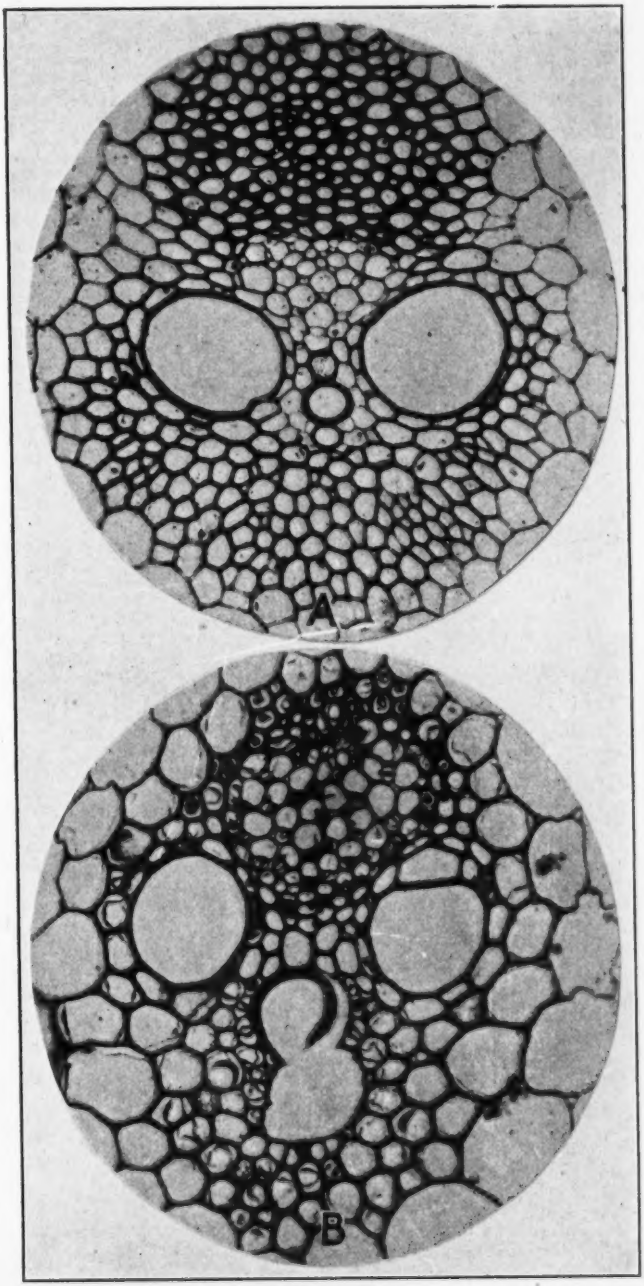


FIG. 3.—Stem bundle with large sclerenchyma cap. × 200. *A*, outer bundle parenchyma; *B*, sclerenchyma cap; *C*, sieve tube; *D*, companion cell; *E*, large pitted vessel; *F*, bundle sheath; *G*, protoxylem; *H*, sclerenchyma cap of xylem pole of bundle; it is poorly developed compared to the cap on the phloem pole.



A.—Large bundle from the more peripheral region of the internode. × 200
B.—Large bundle from a leaf sheath. × 350

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cells, the wall of the vessel next to the sheath cell is not pitted. The tissue between the two large vessels is composed of parenchyma interspersed with narrow vessels (pl. 3, B). The latter are porous and have reticulate thickenings on the side walls. The phloem forms an oval mass of tissue composed of sieve tubes and companion cells. The protophloem lies farthest to the outside; its walls are swollen and somewhat disorganized. This condition is, however, more strongly marked in the protophloem of the leaf (pl. 4, B), and in the stem bundles of Zea (pl. 25). The cells of the bundle sheath are in direct contact with the protophloem; the other phloem cells, however, are separated from the sheath by a layer of small, elongated parenchyma cells. A similar band of parenchyma, one or two cells wide, separates the phloem from the xylem.

The bundle sheath forms a layer of cells which are devoid of intercellular spaces. The cells are elongated, thick-walled and sparingly pitted. The end walls are pointed, except at the junction of xylem and phloem: here the sheath cells not only have a shorter vertical diameter, but the end walls are also nearly transverse.

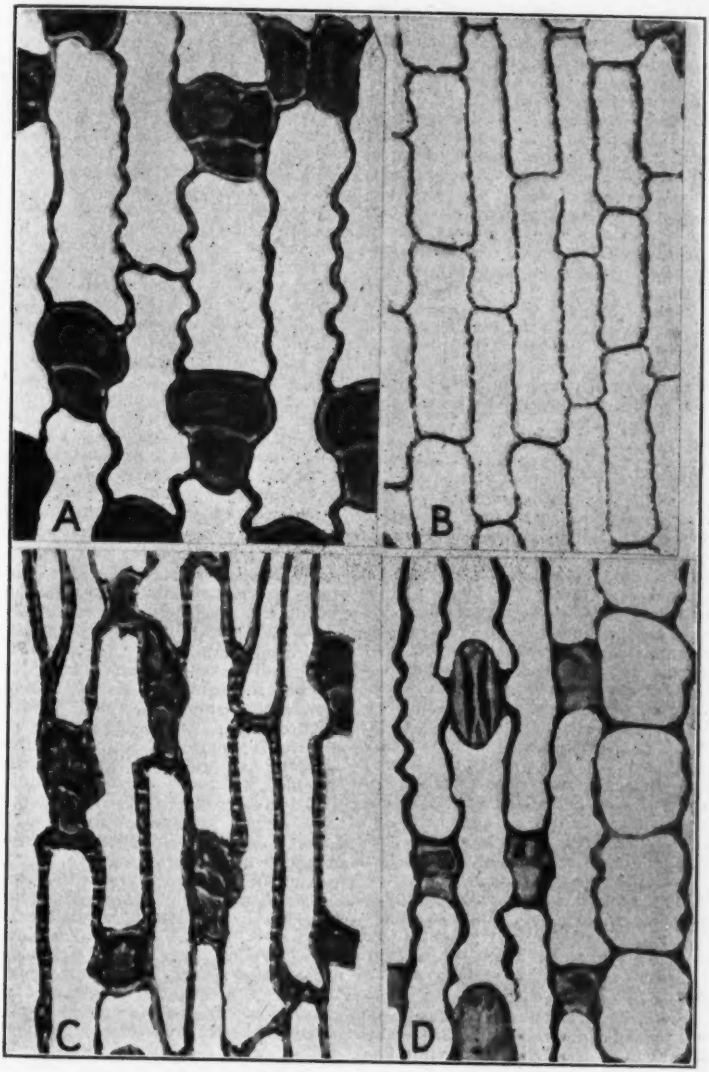
The bundles follow a longitudinal course approximately parallel to each other except in the node where a number of them branch or bend abruptly and move toward the periphery (pl. 1, A). Many of the bundles, however, pass on to the next internode with but slight deviation from their former course. Nevertheless the structure of all bundles is altered when they enter the node.

The peculiar appearance of the nodal bundles when viewed under low power is produced by the parenchyma adjoining the sheath. The parenchyma cells bordering the sheath are greatly enlarged radially and arranged around the bundle in stellate fashion (pl. 6, A). This same arrangement of the parenchyma is often found in the cells adjoining the protoxylem. The sclerenchyma cap of the phloem pole of the bundle is greatly enlarged; the walls of the cells are very thick and strongly lignified. The sclerenchyma cap of the xylem pole of the bundle, however, has practically disappeared. The cells of the bundle sheath in this region are lignified, but the walls have remained thin. The nodal bundles lack the protoxylem lacuna. There is a general increase in the number of protoxylem elements which extend upward and come in contact with the phloem. Occasionally the radial row of protoxylem cells is topped by a tangential band of annular or spiral elements, connecting the two pitted vessels. The latter also have a tendency to increase in number; the supernumerary cells, however, are smaller and less regular. The phloem shows on the whole an appreciable quantitative increase over that found in the internode. The phloem cells, however, have a tendency to become lignified (pl. 6, A), and nonfunctional. The extent of lignification varies, but seems to be in direct proportion to the size and age of the bundle.

As the vascular bundles leave the node and enter the Keimring, they change back to their old form. The stellate parenchyma disappears, the number of pitted vessels is reduced to two, and the protoxylem lacuna is formed again. Changes in the vascular sheath include the development of a cap on the xylem pole of the bundle and a reduction in the size of the cap on the phloem pole. The phloem shows little or no alteration. There is still a great deal of lignification, which is here sometimes more pronounced than in the nodal region.

Before the bundles definitely pass out into the next internode they traverse a region which yields more readily to the sectioning razor. This region is the intercalary meristem from which elongation of the internode takes place. The vascular bundles show surprisingly little lignification, except where the stem is so old that all meristematic tissue has completely matured. The chief divergence from the normal structure consists in a transformation of the sclerenchyma bundle caps into collenchyma and in a general reduction of the lignified cells of the vascular part of the bundle.

PERIPHERAL STEM BUNDLES.—Toward the periphery of the stem the bundles are more crowded, while their form changes from rhomboid to oval with the long diameter radial in respect to the stem (pl. 7, B). The outermost circle of vascular tissue is formed by small bundles which are almost spherical and practically form a solid ring at the periphery of the stem. The oval bundles are actually smaller than those nearer the center of the stem. The apparent increase in size is due to the development of a huge sclerenchyma sheath which is most prominent on the xylem pole of the bundle (pl. 3, A). The phloem is greatly reduced. Its form is no longer oval, with the long diameter in the tangent of the organ, but more or less triangular or trapezoidal, with the narrow part sunken



A.—Epidermis from intercalary region of stem. × 980
B.—Inner epidermis from leaf sheath. × 200
C.—Epidermis of mature stem. × 730
D.—Lower epidermis from leaf blade. × 350

For the sake of clearness the content of the long cells has been blocked out in the negative, so that in the print they appear white

between the large pitted vessels. The protoxylem lacuna has disappeared and the bundle contains thick-walled tracheidlike elements instead. The most peripheral bundles lack the protoxylem and the protophloem and consist largely of sclerenchyma.

In the region of the node these large elongated bundles lose their identity. They appear to break up into small bundles of various configurations, which, together with the peripheral bundles already present in the internode, form a comparatively broad zone of small vascular groups, which, like all bundles of the node, are surrounded by stellate parenchyma (pl. 7, A).

When leaving the nodal region the peripheral bundles become more uniform again. The bundles continue to show large lignified sheaths, but there is no longer that maze of bundle fusion and splitting found in the lower zone. In the upper part of the Keimring the bundles have practically attained the structure and arrangement they possessed in the internode.

Just as the center bundles become temporarily modified when passing through the intercalary meristem, so the peripheral bundles in this region exhibit considerable deviation from the normal structure. The large peripheral bundles upon leaving the root zone and passing through the meristem become so closely crowded that they appear like a honeycomb in which the walls of the comb represent the parenchyma which separates the bundles. The crowding of the bundles is the result of their increase in size, which is produced by the development of a huge collenchyma jacket around a small vascular nucleus (pl. 8). The lignified tissue is reduced to a few protoxylem cells. In the mature stem the same condition exists, except that in addition to the lignified vascular cells there is a thin-walled lignified vascular sheath around each bundle. The sheath is potentially present in the young bundles, but since the cells are delicate and of cellulose, they are not readily distinguished.

SPECIAL BUNDLE STRUCTURES.—At the point of insertion of the leaf sheath the node is traversed by longitudinal traces which, descending from the leaf, penetrate almost horizontally to the center of the stem and thence perpendicularly downward (pl. 15, B). In their horizontal extent the leaf traces deviate from the normal structure. This deviation is shown first in the arrangement of xylem and phloem, which, instead of being distinctly collateral, approaches the amphivasal type. Secondly, the deviation is one of structure. One notices at first glance that the large pitted vessels are wanting and that instead there is an abundance of narrow elements with short articulations and secondary thickenings in the form of very close spirals. In cross section the bundle is typically heart-shaped (pls. 9, A and 10). The xylem is limited to the periphery and distributed in a manner that the narrow spiral elements occupy the tip of the bundle, while at the flanges are found lignified parenchyma cells with scalariform, sometimes reticulate, wall thickenings. The bundle sheath is indistinct and is composed of thin-walled, short fibers of large diameter. The phloem is abundant but lacks the characteristic structure produced by the relative arrangement of sieve tubes and companion cells. It is composed of nearly uniform polyhedral cells, many of which are sieve tubes. The elements of the protoxylem are embedded in parenchymatous tissue, the cells of which later enlarge and partially disintegrate, so that in the mature bundle there is commonly seen a large-celled and lignified lacunate tissue adjacent to the protoxylem.

In addition to these large leaf trace bundles there are numerous small ones which run at right angles to the stem axis. They often surround the ascending leaf traces (pl. 11, A), and frequently fuse with them. The phloem of these small bundles is relatively well developed, while the xylem is often reduced to a single large vessel (pl. 9, B). The bundle is surrounded by a sheath, the cells of which become lignified.

The internodes of the mature stem occasionally contain bundles which deviate from the common structure by the absence of the protoxylem and the reduction of the metaxylem to a single large vessel. The failure of the protoxylem to develop is due to the fact that the bundles were differentiated after elongation in the internode had practically ceased. A second type of deviation is caused by inversed orientation. It is not infrequent to find bundles, especially near the periphery, which have the phloem developed in the direction of the center of the stem, whereas the adjacent bundles are oriented normally, that is, the phloem facing the periphery.

RELATIONSHIP OF THE LEAF TRACES.—The large number of traces entering a leaf and the very complex nodal structure make it impossible to follow the course of the vascular

bundles, except in the meristematic region of the stem apex. Longitudinal sections through such a region show numerous bundles passing from the stem into the leaves. The largest of these bundles pass gradually from the periphery of the stem to the center, and from here, in a curve with the convexity upward, out into the leaf (pl. 12, A and B). The smaller bundles penetrate less deeply, and the smallest remain near the periphery. In regard to the length of the individual leaf traces the same sequence is observed, that is, the largest bundles are also the longest and pass independently through about eight internodes; whereas the smaller bundles are correspondingly shorter, and the smallest cortical bundles terminate in the same internode. Strasburger (*16, p. 328*) found in Zea that the longest leaf traces pass through six internodes. In the sugar cane, the writer has followed a number of the large traces through seven and even eight internodes; their absolute length, however, he was unable to determine with certainty.

The course of the leaf traces is still further complicated in that they describe a spiral in their passage through the stem. For this reason it is very difficult to follow the entire length of a trace in longitudinal sections. This peculiar course of the traces is also responsible for the crossing of traces, which is so noticeable in radial sections (pl. 12, B). The traces of the higher leaves will cross those going to lower leaves and the crossing will be the more striking the thicker the stem and the closer the leaves. In the mature stem, as has been noted, the bundles run approximately parallel to each other, but this is accounted for by the fact that the length of the internodes has increased extremely, and since the large traces pass through many internodes the seemingly parallel course of the leaf traces closely approximates the real situation.

Further complications of the course of the vascular bundles are seen in the node in connection with the vascular supply of the roots and the axillary bud. But, whereas the root connections are derived mainly from the peripheral zone of bundles, the numerous traces going to the bud anastomose with the bundle system of the entire cross section of the stem.

This net of horizontal traces (pl. 11, A) may serve in part other purposes besides the connection of the buds and roots with the vascular arteries of the stem and the leaves. Since there is located above the Keimring the intercalary meristem, where active growth takes place, these numerous horizontal bundles may serve to conduct food material to this meristematic zone, as suggested by Haberland (*7, p. 338*).

THE LEAF SHEATH

A cross section through the middle of the sheath, about midway between sheath and blade joint, shows radial rows of vascular bundles, embedded in fundamental parenchyma (pl. 13, D). The cells of the latter, however, are mostly broken down, so that the vascular bundles are actually separated radially by large air cavities. The vascular bundles lie closer to the outer epidermis (morphologically lower surface) than to the inner one (pl. 14, D). There are bundles of two or sometimes more different orders of size alternating with one another. The large ones occupy approximately the center of the sheath in a cross section, while the smaller ones are close to the outer epidermis and may even touch it. The large bundles frequently consist of two or even three superimposed groups (fig. 4) connected by bands or caps of sclerenchyma. They have the typical shape described for the bundles of the stem, except that in the sheath the phloem is more strongly developed (pl. 4, B). The entire bundle is surrounded by a sclerenchyma jacket which is a continuation of the sheath of the stem bundles. The smallest bundles, which always lie closest to the outer epidermis, consist largely of a mass of sclerenchyma inclosing a few vascular elements.

The parenchyma cells which form the radial prolongation of the bundles in the direction of the inner epidermis (pl. 15, E), show a more or less definite arrangement and terminate in a small mass of sclerenchyma (pl. 14, D), which is separated from the inner epidermis by a row of narrow, parenchymatous cells.

The base of the sheath, also called the sheath-joint (pl. 1 B and pl. 13, A), is swollen and lacks the projecting veins of the sheath proper. Structurally it reflects the molding influence of its location which demands great elasticity of the tissues coupled with late maturation. Increase of flexibility is attained by transformation of the sclerenchymatous elements of the bundle into collenchyma, while greater strength is at the same time afforded by the disappearance of the large air cavities, and the development of small-

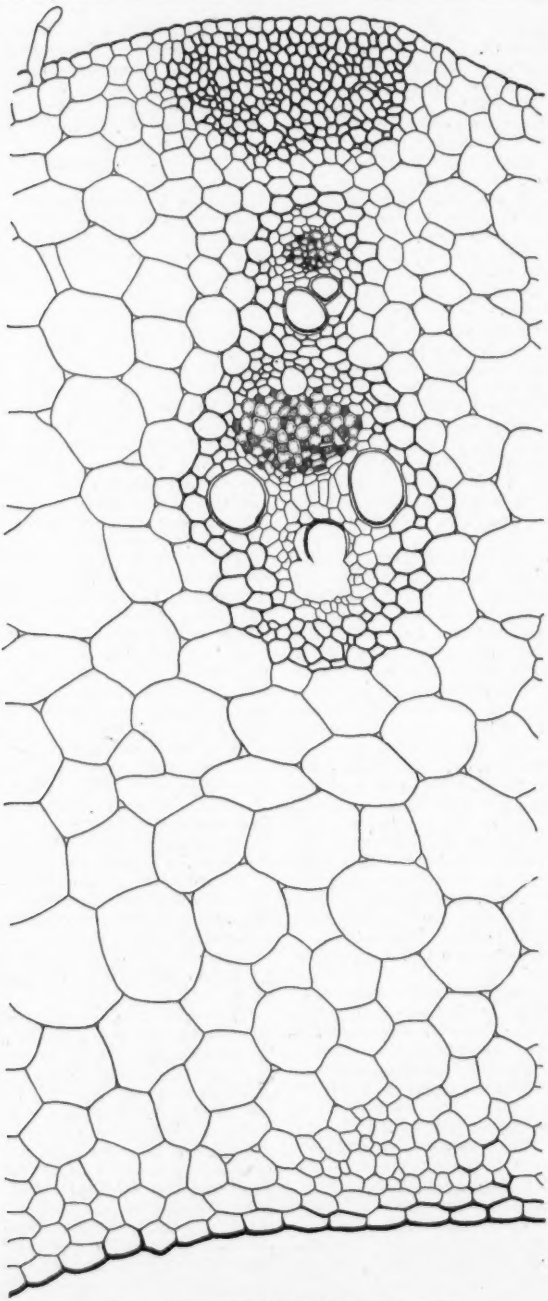


FIG. 4.—Cross section through leaf sheath. ×200

celled parenchyma instead. A cross section through the sheath joint just above the insertion on the stem (pl. 15, D), shows an increase in the number of bundles over that of the sheath proper, and their partial radial displacement. As a result of these changes, no longer are there rows of superimposed bundles, but more or less definite tangential bands, with each band containing bundles of a different magnitude. The bundles of the inner band, that is, the ones closest to the inner epidermis, are the largest and most widely spaced, and are often compound. They constitute the large leaf-traces which, as may be seen from a study of the course of the vascular bundles, penetrate far into the stem and extend to a depth of several internodes. Often, just before passing out into the leaf, two or even more bundles fuse more or less completely, which accounts for the presence of the compound bundles in the sheath joint. The second circle contains smaller bundles which penetrate less deeply and are much shorter in vertical extent. Finally, the outer circle is formed by small groups consisting mostly of collenchyma (pl. 13, E).

The structure of the large bundles resembles somewhat that of the bundles found in the intercalary zone of the stem. On the xylem pole of the bundle there is a large collenchyma cap which is separated from the bundle itself by a single layer of parenchyma. The xylem is composed of spiral elements which partly surround the phloem. The smaller bundles have increasingly larger amounts of collenchyma, and the smallest may be exclusively collenchyma. Both below and above the sheath joint the collenchyma terminates and is replaced by sclerenchyma. The supernumerary bundles gradually disappear, and between the radial rows of bundles the parenchyma exhibits partial disintegration with the formation of the typical air cavities.

In the growing cane, the lower, older leaves are progressively shed, constituting the so-called process of "self cleaning" typical of this group of cane varieties. In the mature plant there is a naked stalk with a tuft of large leaves at the apex. The leaves become detached from the stem in the region of the sheath joint, which, as previously discussed, is anatomically different from the other regions of the leaf, in that the sclerenchyma fibers are replaced by the much softer collenchyma. The sheath joint may in this connection be considered the abscission zone of the leaf.

The apex of the sheath also undergoes modifications before it merges into the blade. As the leaf sheath approaches the blade joint it becomes narrower and thicker, thereby crowding the vascular bundles more and more together. The air cavities between the bundles disappear, being replaced by parenchymatous tissue. The sclerenchyma groups near the outer epidermis gradually enlarge, while the vascular bundles at the same time move closer to the center of the sheath. The sclerenchyma groups of the inner epidermis also enlarge considerably and gradually unite into a solid tangential band (pl. 15, C). While these changes are going on, the tangential band of sclerenchyma becomes separated from the inner epidermis by a progressively widening band of parenchyma (pl. 15, C).

The part of the sheath just above the insertion of the ligule has been referred to as the blade joint (pl. 1, C and fig. 5). The flanges of this joint are brownish in color, strongly pubescent, and of a soft texture. The vascular tissue in this region resembles that found in the sheath joint. As in the latter the large bundles inclose much collenchyma, whereas the xylem consists entirely of spiral vessels. The vascular tissue of the median blade joint, which becomes continuous with the bundles of the midrib of the blade, does not show the modifications exhibited by the flanges. If there are any noticeable changes they are in the direction of increased lignification and the formation of ever broadening lignified bundle caps. In the transition region from the median part of the blade joint to the flanges, there is noticeable a gradual transformation of the sclerenchyma into collenchyma; one frequently observes bundles which have thick collenchyma caps, of which half of the cells consist of lignified sclerenchyma.

The inner epidermis of the sheath differs fundamentally from the outer epidermis in that the cells of the former are large, uniformly rectangular, and of one type, since the short silicified cells are wanting (pl. 5, B). Hairs and stomates are only sparingly developed, except just above the insertion point of the ligule, where they form a dense fringe. This same type of epidermal cell is found on the inner surface of the ligule (the side which is opposite the stem). The cells just below the ligule are broad and more or less irregular, whereas above the ligule they show a transition to the cells of the leaf blade, and become long, narrow, and thick walled.

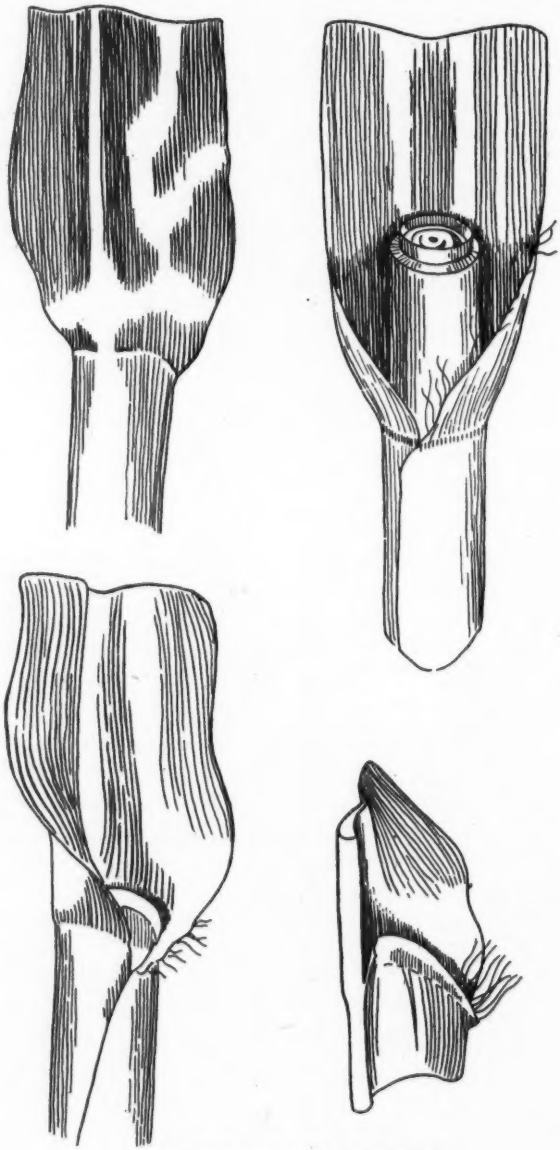


FIG. 5.—Four views of blade joint and ligule

The cells of the outer epidermis of the sheath resemble those of the stem (pl. 13, C). They are very thick-walled, undulated and pitted. The short cells are very conspicuous and in specialized regions, as for example at the blade joint, they are more numerous than elsewhere. The cells above the veins are always long and narrow and possess a nearly closed lumen; those between the veins are broader, but otherwise structurally the same. The epidermal cells of the sheath joint and the flanges of the blade joint are thin-walled and irregular in shape. Stomates and hairs are well developed. The former occur in longitudinal rows next to the veins. The hairy covering is most extensive above the sheath joint (pl. 14, B) and at the flanges of the blade joint. Above the sheath joint the number of hairs decreases greatly, coincident with a change in their character. Instead of the extremely long and delicate hairs which are characteristic of the regions referred to above, short and strongly lignified setae interspersed with two-celled appressed hairs are found.

THE LIGULE

The ligule contains no vascular tissue but is made up altogether of elongated parenchyma cells which are comparatively thin-walled and of cellulose. Both inner and outer epidermis are suberized. The outer epidermis, the one next to the sheath, is covered with numerous long hairs which, arising at the base of the ligule, are adnate to the epidermis (pl. 13, C) and become free at the apex where they form a minute fringe. The hairs are commonly strongly lignified at the base. The outer epidermal cells are fairly thick-walled and slightly undulated; the inner ones are broad, especially at the base, the walls are thin and more undulate than those of the outer epidermis. All epidermal cells are of one type, silicified cells as well as stomates being absent. The ligule has a protective function in that it prevents the entrance of water between stem and sheath.

THE LEAF BLADE

The tissues of the blade are continuous with those of the sheath and in general show similar structure. Differences such as are found are consistent with the chief rôle of the blade as an organ for assimilation and aeration.

The vascular bundles are of three ranks (pl. 16, C): (*a*) small round bundles, (*b*) medium large oval bundles, and (*c*) large rhomboid bundles. The small and medium large bundles alternate with one another, whereas the large bundles, corresponding with the more prominent veins of the leaf, appear at wider intervals. The large bundles are always bound on either side by a small round bundle. The small bundles are always close to the lower epidermis, whereas the other two types occupy the middle of the blade. In the region of the midrib (pl. 16, B) however, a layer of colorless parenchyma is interpolated between the bundles and the upper epidermis. This layer increases in thickness, pressing the vascular tissue gradually toward the lower surface, while the scattered sclerenchyma groups on the upper surface unite to form a solid band. The resulting tissue area has the form of a half moon, or triangle, the lower surface of which is studded with vascular bundles, while the upper surface, which contains no bundles, is reinforced by a solid layer of sclerenchyma.

The bundles of the blade differ primarily from those of the stem in that they possess a chlorophyll-bearing bundle sheath and an inner sheath in the region of the phloem (pl. 1, D). The vascular tissue itself differs in the lack of a protoxylem lacuna except in the very large veins, and in a greater development of the xylem (pl. 17, A).

The chlorophyll-bearing bundle sheath, which forms a continuation of the sclerenchyma sheath of the stem and leaf sheath bundles (pl. 17, B), is made up of uniform large and spherical cells which vertically have the form of short cylinders (pl. 18, B, C). In the region of the phloem pole of the large bundles the sheath cells become smaller or may even be wanting. Through the gap, which is formed thereby, the fibers of the inner sheath become continuous with the fibers of the hypodermal sclerenchyma. But while the fibers of the inner sheath appear, in some instances at least, ontogenetically the same as those of the hypodermal sclerenchyma, they differ structurally in that the former possess numerous pits. In small bundles, where the cells of the inner sheath are undifferentiated as yet, the phloem is separated from the chlorophyll-bearing sheath by a layer of parenchyma cells, which differ from the phloem cells only by their larger size.

The xylem of the large bundles consists of two large vessels connected by a more or less extensive band of small pitted elements, most of which are narrow vessels. In the small bundles there are only a few rela-

tively large pitted vessels which border directly on the phloem (pl. 17, A.) As is usual, however, phloem and xylem cells do not communicate by pits. The phloem is structurally similar to that found in the bundles of the leaf sheath. This is especially true of the bundles of the midrib (pl. 18, A). The sclerenchyma cap of the bundles of the midrib abuts directly on the epidermis.

Transverse bundle connections are frequent. They are in the nature of narrow branches which run somewhat diagonally, or at right angles, between two veins and effect union with the latter. As the leaf becomes narrow there is a gradual reduction in the number of bundles. The bundles which are to drop out either fuse with other bundles or they become so completely reduced that only a few phloem and xylem cells remain and finally only elongated parenchyma.

The chlorophyll-bearing parenchyma of the leaf forms a more or less concentric ring around the bundles, except when the latter are so large that they practically fill the area between the two epidermis. In the midrib the chlorophyll-bearing parenchyma is found largely between the small bundles. The cells are small, irregular, and filled with numerous chloroplasts, the color of which is darker green than that of the plastids found in the sheath cells. This apparent difference in color is probably due to the fact that in the spongy parenchyma the chloroplasts are more crowded than in the sheath where they are few in number and much larger individually. In fixed material the large chloroplasts have a tendency to clump together in narrow crescent-shaped bands, (pl. 17, A). Adjacent to and partly surrounding the spongy parenchyma are large colorless cells, which have some peripheral protoplasm and a watery sap. They probably serve for the storage of water.

The cells of the epidermis are brick-shaped with the largest diameter in the direction of the long axis of the organ. The walls of the cells are strongly undulated, thickened and lignified (pl. 18, D). In addition to the long cells there are found, just as in the epidermis of the stem and leaf sheath, short cells singly or in pairs (pl. 5, D). One component of the pair is commonly thick-walled and strongly silicified. The epidermal cells above the veins differ from the normal type in being greatly elongated and often so thick-walled that there is hardly any lumen. The upper epidermis has in addition to the two types of cells mentioned, the so-called "bulliform" cells (pl. 16, A), which form longitudinal bands and alternate with strips of ordinary epidermal cells. The bulliform cells are much larger than the other cells of the epidermis and differ further in that their walls remain thin and of cellulose. They are continuous with the inner part of the leaf through the large colorless cells rereferred to above. The bulliform cells differentiate only after the leaf begins to unroll, but then they increase in size rapidly and become slightly raised above the level of the other cells.

The margin of the leaf is serrate; the teeth are formed by stiff setae of somewhat triangular form. The rows of epidermal cells next to the margin are thick-walled, colorless, and almost devoid of content.

Except for the structural deviation exhibited by the margin of the leaf the lamina is remarkably uniform. The veins, which occur at regular intervals, divide the blade into narrow bands of tissue composed of rows of regular epidermal cells, alternating with rows bearing either stomates of hairs. The latter exhibit two distinct types: short one-celled bristles, which attain a larger size at the lower leaf surface, and two-celled appressed hairs. There are commonly three rows of stomates between two veins, but on the upper leaf surface they are less numerous and are commonly limited to a single row at each side of a large vein.

The stomates are phaneroporous, lying in the same level with the epidermal cells. They are always found in longitudinal rows in such a manner that a stomate alternates with an epidermal cell. The mature stomate is formed by two guard cells and the adjacent subsidiary cells (pl. 19, A, C, D, and E). The latter become much larger than the guard cells, extend deeper radially and their outer walls slope slightly in the direction of the guard cells. The guard cells are wider at the ends than in the middle region of the cell. In longitudinal sections it will also be noticed that both tangential walls of the guard cells, but especially the inner walls, are deeply constricted in the middle, which further tends to narrow the lumen of this part of the cell. The pore of the stomate is elongated lozenge-shaped, its lateral walls being straight and parallel, or sometimes gently curved. The stomatal movements are correlated with this peculiar construction of the guard cells. Schwendener

(*15*), who fully investigated the stomatal movements of the grasses, explains the opening and closing as follows: Since the middle portion of the guard cell is narrow and the tangential walls greatly thickened, any change in the osmotic pressure of the cell would be incapable of causing movement in this portion of the wall. The enlarged ends of the guard cells, however, have thin walls which will respond to turgor changes. When there is an increase in cell turgor they cause the pore to open, whereupon the thickened middle portions of the guard cells are passively drawn apart leaving a slit with parallel sides.

THE ROOT

The root differs from the stem by two structural features, the possession of a root cap, and the radial arrangement of the bundles. The root cap occupies the extreme tip of the root. It is often colored red, which, ordinarily a normal condition, sometimes is of pathological significance.

A cross section of a young root (pl. 20, A) shows a cylinder of vascular tissue limited on the inside by a well developed pith, on the outside, by the endodermis and cortex. The pith is of uniform nature and is made up of large spherical cells, interspersed with small intercellular spaces. The cells are elongated vertically and have a tendency to become larger toward the center. Occasionally there are found scattered in the pith small, thick-walled cells similar in size and shape to the interstitial tissue of the stele (pl. 21, A). The cortex possesses on the inner and outer periphery, layers of small and specialized cells. Those at the outer periphery are narrow, elongated and thick-walled and form in their entirety a distinct sclerenchymatous cylinder. The rows of cells at the inner periphery consist of regular small square cells between which are found rhomboid intercellular spaces (pl. 20, B). The larger part of the cortex consists of loosely packed parenchyma cells which later disintegrate with the formation of large air chambers.

The epidermis is made up of fairly uniform and thin-walled cells which vertically have the form of short cylinders. Many of the epidermal cells have grown out into hairs which remain attached to the root long after they have ceased to function. Beneath the epidermis is the exodermis, a layer of thin-walled parenchymatous cells, which are larger and more elongated than the cells of the epidermis. The walls of these cells are suberized, and their inner tangential surface is always thickened.

The endodermis as shown in Plate 22, B, C, and D consists of a single layer of cells connected uninterruptedly with one another. The individual cell is a vertically elongated four-sided prism with horizontal ends. The radial walls slightly exceed in length the tangential ones, though sometimes they are equal. The cells are thin-walled in their meristematic condition, but upon maturing the inner tangential walls and parts of the radial ones become greatly thickened and lignified. The Casparian strips are seen in young material but even here only with difficulty; later they become masked by the development of secondary wall thickenings. The inner tangential walls of the epidermal cells are distinctly pitted and possess peculiar protuberances which extend into the lumen of the cells. The apical region of such protuberances as shown by Klinge (*9*) and Bremekamp (*3*) contains silica.

The vascular tissue consists of alternating masses of primary xylem and phloem which occupy different radii (pl. 23, A). The xylem plates do not reach the center. The latter is occupied by a parenchymatous pith at the periphery of which there is a ring of large pitted vessels. (Pl. 20, A and pl. 22, A.) Outside of this there is a ring of alternating xylem and phloem groups. In young roots (pl. 24, A) there are almost always eight large vessels; in older and thicker roots (pl. 21, A) this number is much larger. The clusters of protoxylem and phloem are very numerous (pl. 23, A), a result of the absence of secondary growth which is so important a factor in the development of the roots of the dicots. In the thin lateral roots and in the rootlets of seedlings, the xylem plates are few in number and meet in the center; a pith and the large pitted vessels are often wanting (pl. 24, B). The groups of xylem are separated from the endodermis by a single-layered pericycle, but in the small roots the xylem cells occasionally border directly on the endodermis. Between the groups of primary xylem and phloem is interstitial parenchyma (pl. 23, B and fig. 6). Cells of the same tissue also separate the protoxylem and phloem from the large vessels, then surround the latter and form a pcripheral sheath around the pith. The character of these cells, however, differs with the region where they occur. Thus the

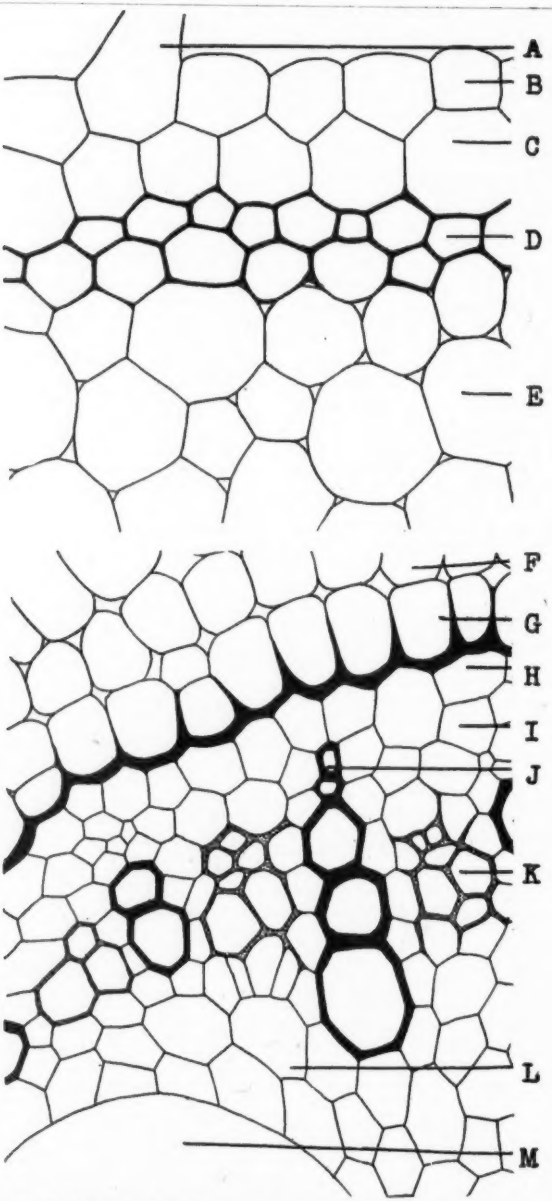


FIG. 6.—Partial cross section of young root. × 510. A, root hair; B, epidermis; C, exodermis; D, sclerenchyma tissue; E, cortex (only a few cell layers have been drawn—the actual cortex is broader); F, inner cortex; G, endodermis; H, pericycle; I, interstitial parenchyma; J, protoxylem; K, phloem group; L, parenchyma between protoxylem and large pitted vessel; M, large pitted vessel (still in meristematic condition)

cells which separate the groups of protoxylem are elongated, rectangular, and profusely pitted; those at the periphery of the pith are sclerenchymatous pointed, and sparingly covered with narrow slanting pits. The thickening and lignification of these sclerenchymatous cells begin soon after the protoxylem is formed and while the large vessels are still meristematic. The first of these cells to become lignified are those adjacent to the pit and the process of maturing is thence continued in centrifugal direction (pl. 20, B, and pl. 23, A). The order of development differs therein from that of the protoxylem, which is in the direction of the root axis.

The structure of the protoxylem of the root is peculiar in that it lacks annular and spiral elements. The first-formed cell is a narrow element with scalariform or spiral-reticulate wall thickening; the second protoxylem cell is similar though often much larger than the first one and is usually scalariform. Quite frequently the larger of the two protoxylem cells develops first and only later a small cells is added centrifugally.

The groups of phloem alternate with the protoxylem. They are always small and rarely contain more than eight elements. The cells of the phloem are pentagonal or square in cross section and are readily recognized by the slightly thickened refractive walls (pl. 23, B).

In old roots all the cells of the vascular tissue, with the exception of the phloem, become lignified and thick-walled. Similar changes take place in the layer of cortical cells which border on the endodermis. Concomitant with these changes is a disintegration of the cortical tissue. The radial strips of tissue which remain are attached to the exodermis on the one hand and the sclerotic layer of inner cortical cells on the other (pl. 21, B). Occasionally certain cells of the inner cortex do not lignify so that the radial strips of cortex become attached to the endodermis directly.

ONTOGENY

Material for study was selected from the apical region of a large, actively growing cane. The stem portion measured 9 cm. and contained 14 internodes of which the upper 10 were crowded into a space of only 10 mm. (fig. 7). The relative length of the leaf sheath and blade of the first 14 leaves follows on page 218.

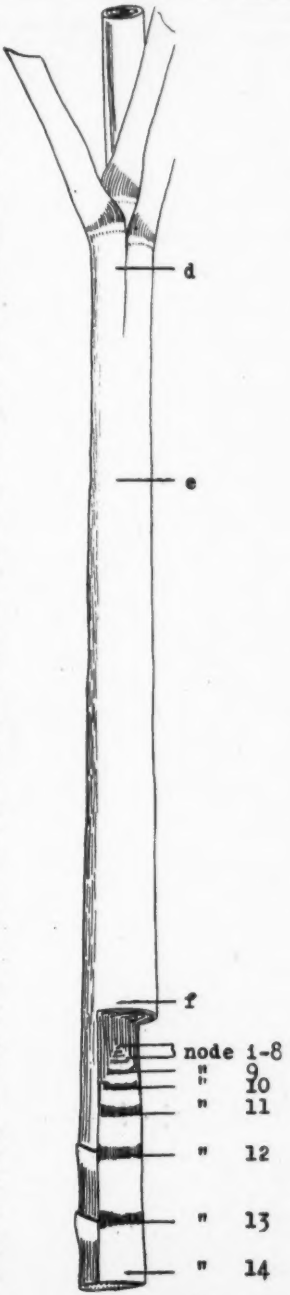


FIG. 7.—Upper part of sugar cane with leaves cut off and stem split open so as to expose the growing point and show its position in regard to the leaves. Nodes 14, 13, and 12 have leaf sheaths already mature, *d* indicates the position of the termination of the leaf sheath of node 11, *e* of node 10, and *f* of node 9. The leaf sheaths of the upper nine nodes are hardly differentiated as yet

Succession of leaves on the stem axis [a]	Length of sheath	Length of leaf blade	Succession of leaves on the stem axis [a]	Length of sheath	Length of leaf blade
	Cm.	*Cm.*		*Cm.*	*Cm.*
First	------	----	Eighth	1.2	120
Second	------	----	Ninth	3.0	155
Third	------	----	Tenth	22.0	185
Fourth	------	----	Eleventh	31.0	185
Fifth	0.1	32	Twelfth	33.0	165
Sixth	.2	50	Thirteenth	34.0	190
Seventh	.2	90	Fourteenth	33.0	186

[a] The first four leaves are not yet differentiated into sheath and blade.

Beginning with the twelfth leaf (or the youngest leaf, the blade joint of which is already superficially exposed) the length of the sheath remains practically the same and fluctuates slightly about a mean of 33 cm. While the leaf sheath is rather belated in its development the blade enlarges rapidly as soon as it is cut off from the apical meristem. Thus, a blade about 1 meter in length (at that stage still rolled in the bud) is borne by a sheath only 4 mm. long. However, although unequal at the start, blade and sheath attain their maximum length practically at the same time with the blade slightly in the lead.

The development and differentiation of the different tissues of the stem follow in sequence the external differentiation. The first eight internodes of the stem, which have a total height of a few millimeters only, show the early beginning of tissue differentiation. The growing point is made up of a thin-walled meristem, the cells of which are in a state of active division (fig. 8). Gradually certain of the cells begin to enlarge, setting off thereby small groups of cells which continue to divide actively. These groups constitute the primordia of the vascular bundles, and within them differentiation proceeds until all the components of the bundle are formed.

The protoxylem differentiates first. It is made up of a few narrow, but greatly elongated elements, which have secondary thickenings in the form of rings. The third protoxylem element is usually a spiral vessel. While differentiation of the protoxylem proceeds in centrifugal direction, there appear on the opposite pole of the group the first elements of the phloem recognizable by the refractiveness of their thickened walls. Between xylem and phloem there is an actively dividing tissue which sometimes takes on the appearance of a typical cambium and from which all other xylem and phloem cells are formed.

Not all bundles in a cross section of the growing point show the same degree of differentiation. A certain number of them are larger and contain a greater number of protoxylem cells. The larger xylem elements have closely ringed wall sculpture and comparatively short articulations. In a cross section just below the insertion of the young leaf these bundles are seen to bend more or less abruptly and move toward the periphery, where they enter the leaf. They constitute the large leaf traces which already in their very young condition foreshadow the structure characteristic of the mature traces. The peripheral stem bundles are much smaller than those nearer the center with many of them just in the state of procambial differentiation.

Cross sections through the tenth internode (just 1 cm. below the growing point) show the differentiation of the large pitted vessels. The walls of these cells are still very thin and easily crushed in making a section. The tissue surrounding the xylem and phloem is composed of narrow, elongated parenchyma cells which gradually mature into the bundle sheath. The development of the bundle sheath, however, is very slow and is completed only after all the vascular elements of the bundle are fully formed.

In the twelfth internode the large vessels have developed a secondary wall which shows the pitting characteristic of the mature element. The cross walls between the articulations of the vessels have also disappeared and communication is now established through large pores. As the large vessels complete their development the phloem cells divide longitudinally to form the sieve tubes and the companion cells of the mature bundle.

In the next lower internode the walls of the large vessels have become lignified. The cells of the sheath have ceased division and have begun to thicken their walls, which is especially pronounced in the cells forming the bundle caps.

Finally, in the fourteenth internode, with the lignification of the sclerenchyma sheath, the formation of the protoxylem lacuna and the obliteration of the protophloem, the stem bundle is completely mature. The formation of the protoxylem lacuna may be observed at an earlier stage, but then only in individual bundles.

Hand in hand with the progressive differentiation of the different elements of the vascular bundle goes an increase in the size of the cells. This increase, however, is not so marked in the bundle

itself as in the surrounding parenchyma. The cells of this tissue, at a time when the large vessels are just differentiating, are about 65μ in width. When the bundles are fully developed, the parenchyma cells measure 125μ and may attain even greater dimensions. This increase in size of the parenchyma cells is in a large measure responsible for the enlargement of the stem, since the maximum number of bundles present in a stem is reached long before the latter has attained its final thickness.

The differentiation of the tissues of the leaf sheath has been described by Kobus (*10*). It takes place in the same sequence as in the stem, only it seems to be more rapid. Thus an internode which bears a leaf sheath 3 to 4 cm. long has only a few mature protoxylem elements, while in the leaf sheath the large vessels are already fully developed, although not as yet lignified.

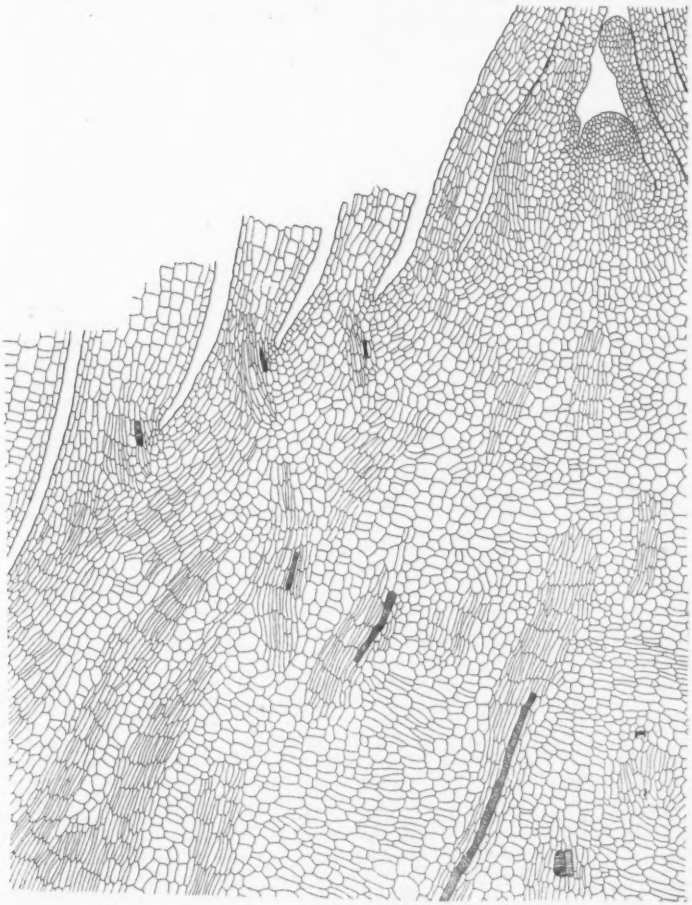


FIG. 8.—Longitudinal section through the growing tip of a large sugar cane. × 225

The phloem of the leaf sheath also develops in advance of that in the stem, since differentiation into sieve tubes and companion cells has already taken place.

The epidermis of the young stem and of the lateral organs is made up of thin-walled rectangular cells (pl. 11, B).

In their further development these cells either merely enlarge as, for example, the cells of the inner epidermis of the leaf sheath and the ligule, or they undergo further modifications. In the latter case each cell divides unequally giving rise to a long cell and a short cell. The long cell elongates still further, while its walls become thick and strongly undulated. The short cell either ceases growth and undergoes silicification, or, according to Pfitzer (*14*), it becomes the primordium of a hair or stomate.

In the development of a hair the peripheral tangential walls of the epidermal cell begin to show marked apical growth. As the cell enlarges, the nucleus divides (pl. 14, A). The binucleate condition exists until the cell has attained approximately its full length; a wall then develops which divides the hair into two more or less equal parts. In the later development, the base of the hair becomes lignified, while in the apical region there is often found a slight brownish precipitate. The first-formed hairs are commonly two-celled. They are always delicate and ephemeral. Short unicellular hairs with thick, lignified walls are characteristic of the more mature organs. In the region of the sheath and blade-joints the unicellular hairs attain a considerable length, which reaches its maximum in the fringes at the base of the ligule.

The short cells of the epidermis next to the veins of the leaf and more sparingly in other regions continue development into stomates. The short cell constitutes the stomate mother cell which by further division gives rise to the two guard cells. The two subsidiary cells of the stomate are formed as lateral outgrowths from the adjacent parenchyma cells. With the formation of the stomatal chamber and the separation of the wall between the two guard cells the development of the stomate is complete. The stomates of the inner epidermis of the leaf sheath differ from those of the stem and leaf blade in that the subsidiary cells are very broad; the guard cells too appear to be slightly different and the stomatal pore instead of being a narrow cleft of even width is more or less oval (pl. 19).

The epidermal cells of the stem remain thin-walled for a long time, even after the vascular bundles are fully developed. Eventually, however, the walls thicken, especially the outer ones, which in addition become cuticularized and finally covered with a layer of wax. The epidermis of the leaf blade and the outer epidermis of the sheath also become thick-walled and lignified.

SUMMARY

The vascular bundles of the sugar cane are of the normal monocot type, rhomboid to oval in cross section and surrounded by a well-marked sclerenchymatous sheath which is enlarged on the phloem and xylem poles into bundle caps. Phloem and xylem are disposed collaterally in relation to each other. Deviations from this arrangement are observed in the node and the lower leaf sheath, since here certain of the bundles show a more or less amphivasal structure. True amphivasal arrangement, however, is found only in the larger traces of the buds. In the lower leaf sheath a pseudo-amphivasal arrangement is sometimes produced by the formation of compound bundles.

The xylem is composed of some protoxylem and two large pitted vessels. The protoxylem forms a short radial row of annular and spiral elements. It is more extensive in the node than in the internode and reaches its maximum development in that part of the leaf traces which runs horizontally in the node. The large pitted vessels of the metaxylem also tend to increase in number as the vascular bundle passes through the node; the large horizontal leaf traces on the other hand lack the large vessels altogether. The internodal bundles, except those near the periphery, and the large bundles of the leaf sheath and the blade possess a more or less conspicuous protoxylem lacuna. The lacuna is absent in the bundles of the node, the peripheral bundles of the internode, and the smaller bundles of the leaf.

The phloem consists of sieve tubes and companion cells. It is most extensive in the bundles of the node and the leaf sheath, but least conspicuous in the smaller bundles of the leaf blade and the peripheral bundles of the internode. Changes in the phloem consist in obliteration of the protophloem, which is most pronounced in the large bundles of the leaf, probably because of the development of an inner fibrous sheath above the phloem cells and in a lignification of certain portions of the mature phloem tissue. Lignified metaphloem is most frequently observed in the large bundles of the node and in the Keimring.

The vascular bundles are surrounded by a sclerenchymatous sheath which is most strongy developed on the inside

and the outside of the bundle, where it forms typical caps. In the peripheral internodal bundles the sheath increases greatly but mostly on the xylem pole, whereas in the nodal bundles the sclerenchyma caps on the phloem pole are most conspicuous. In the bundles of the leaf blade the cells of the sclerenchyma sheath become larger, more regular, and contain large chloroplasts. In addition, the leaf bundles possess a fibrous sheath located between the chlorophyll-bearing sheath and the phloem. The extent of this fibrous sheath is in direct relation to the size of the bundle.

The epidermis of the stem and of the lateral organs is made up of two types of cells—long cells with strongly undulated walls and short silicified cells. The inner epidermis of the leaf sheath and the ligule, however, consists only of long cells. The epidermis of the mature stem is strongly lignified and covered with a layer of wax. The subepidermal cells also become lignified, but thin-walled parenchyma cells are interpolated at intervals. The epidermis of the leaf sheath and the blade bears hairs and stomates of a type common to grasses.

The vascular tissue of the root forms a siphono stele bounded externally by a cortex, internally by a pith. The peripheral cortical cells are suberized, forming a well-marked exodermis. Between the pith and the vascular cylinder is a sclerenchymatous layer which becomes lignified very early, and affords support to the young and delicate vascular tissue of the root. The xylem plates of the root are separated from the endodermis by a single-layered pericycle, though in small, lateral rootlets a xylem cell may border directly on the endodermis.

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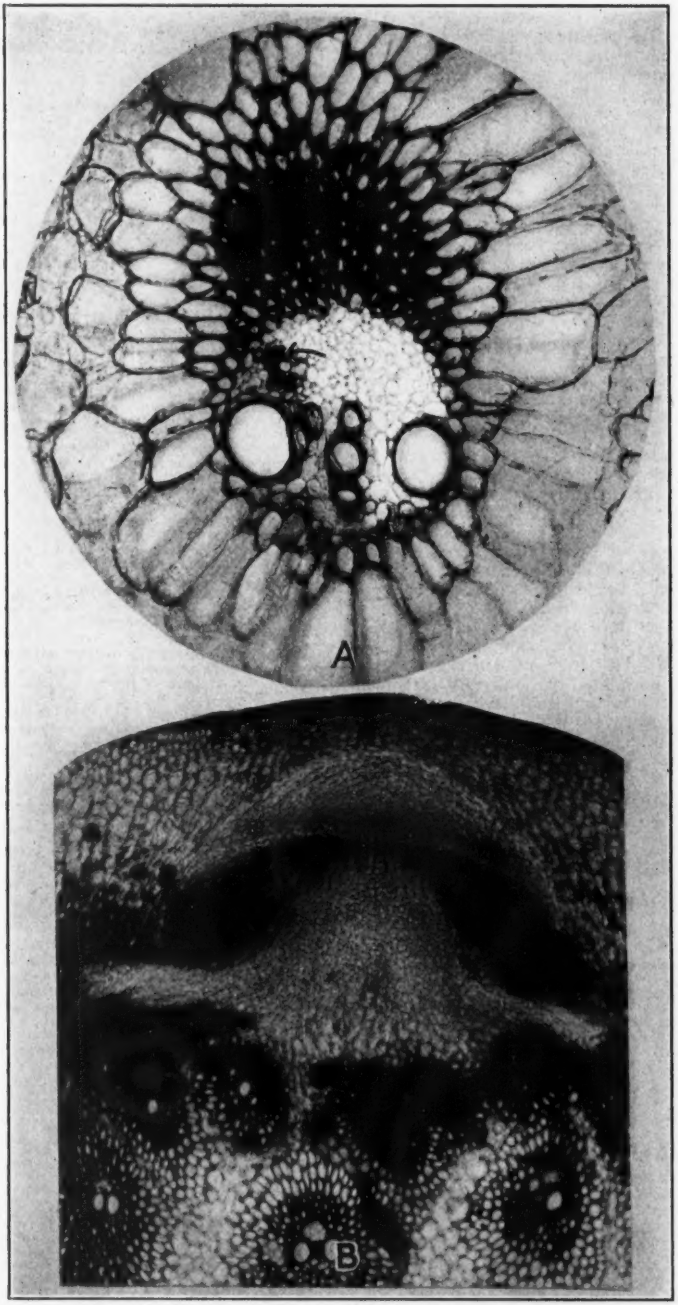
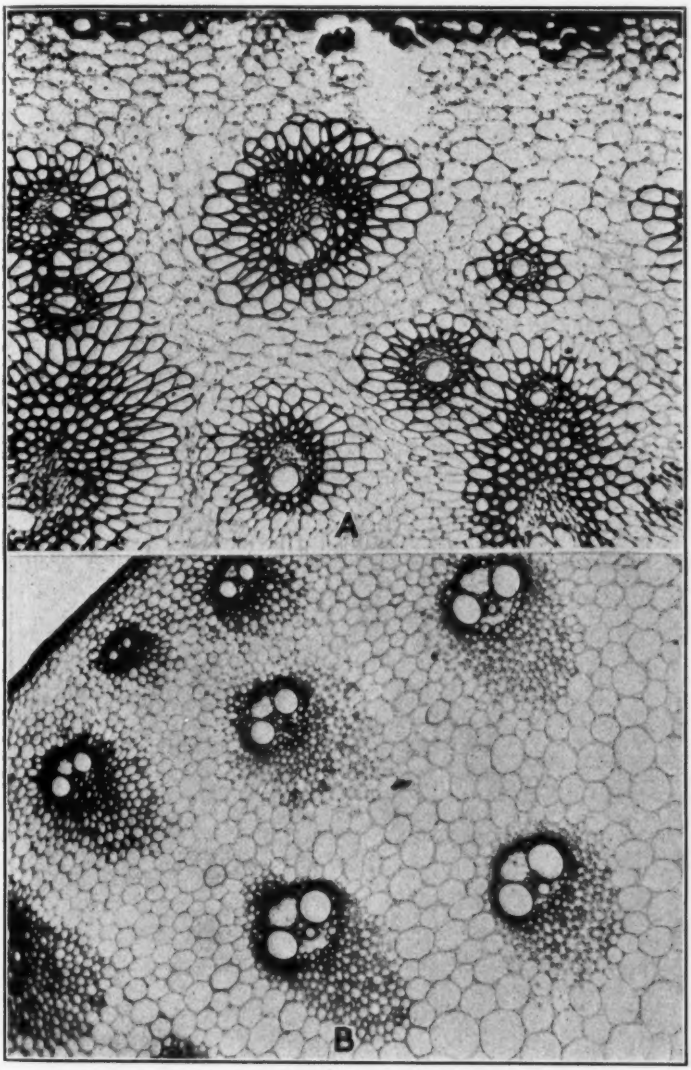
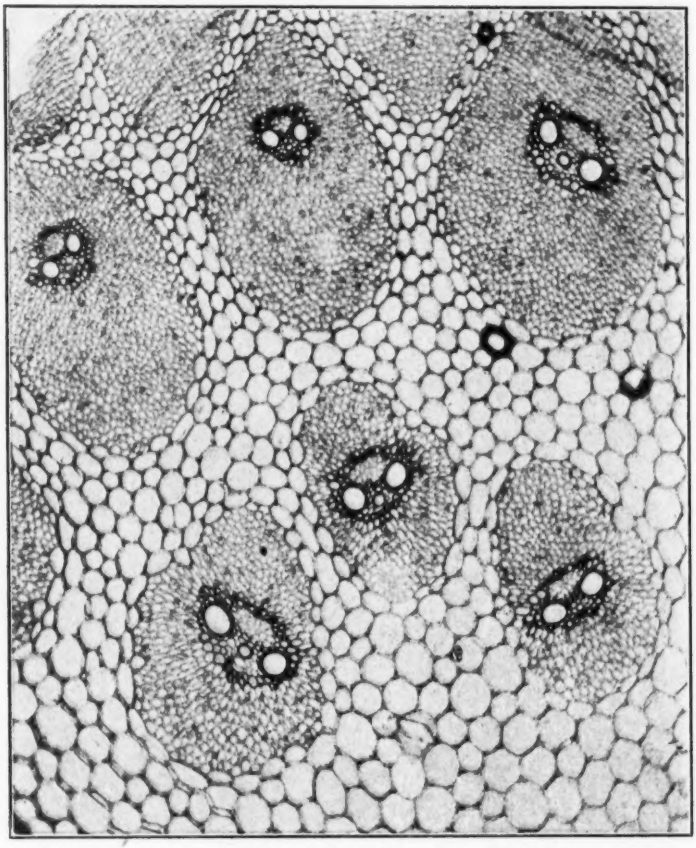


A.—Cross section of mature nodal bundle. Notice the lignification in the phloem (designated by the arrow). × 124

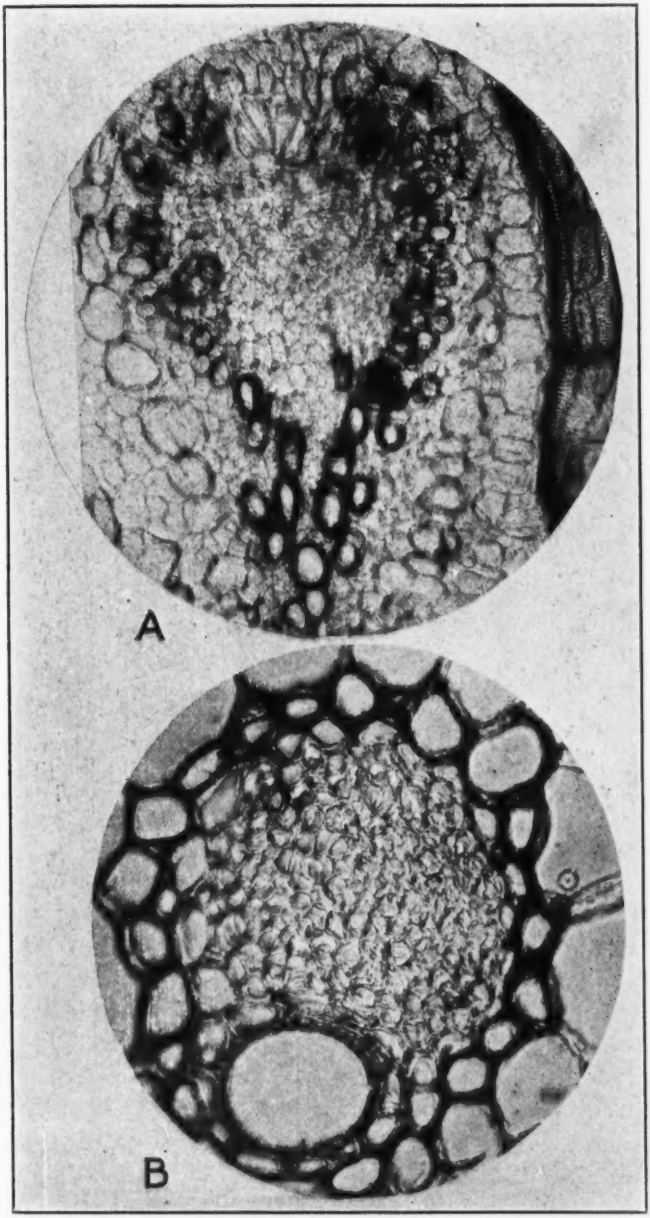
B.—Cross section through the peripheral part of the Keimring showing a root primordium. × 80



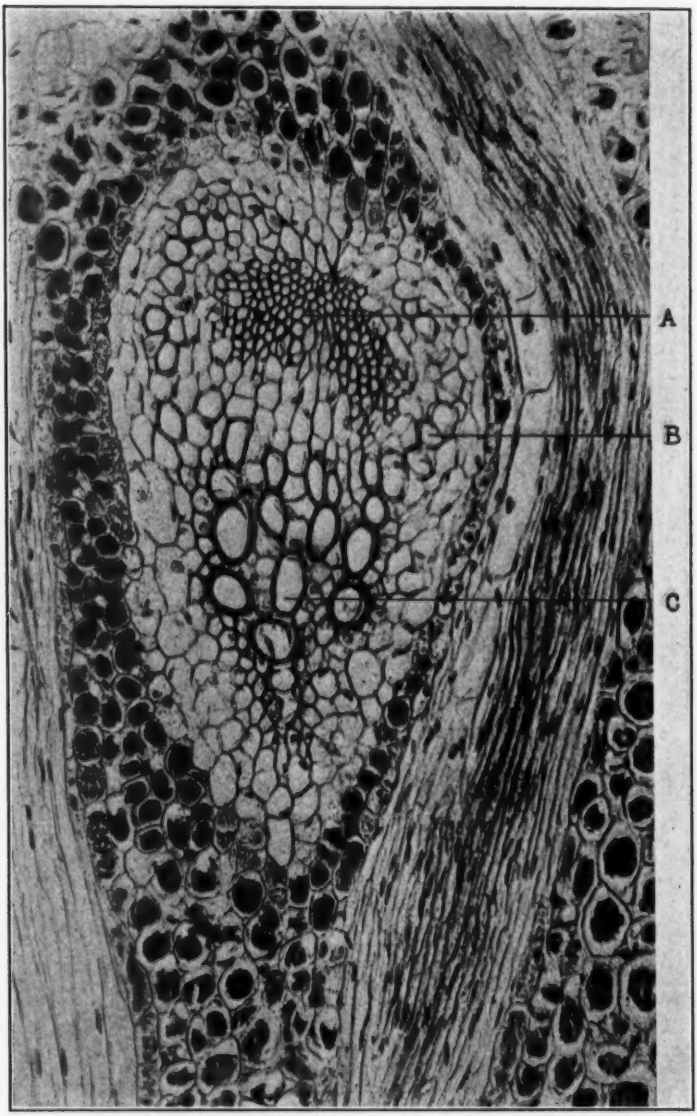
A.—Cross section through outer region of node of mature stem. × 106
B.—Cross section through peripheral part of internode of mature stem. × 85



Cross section through the peripheral region of the intercalary meristem of internode (hand section of fresh material). × 188

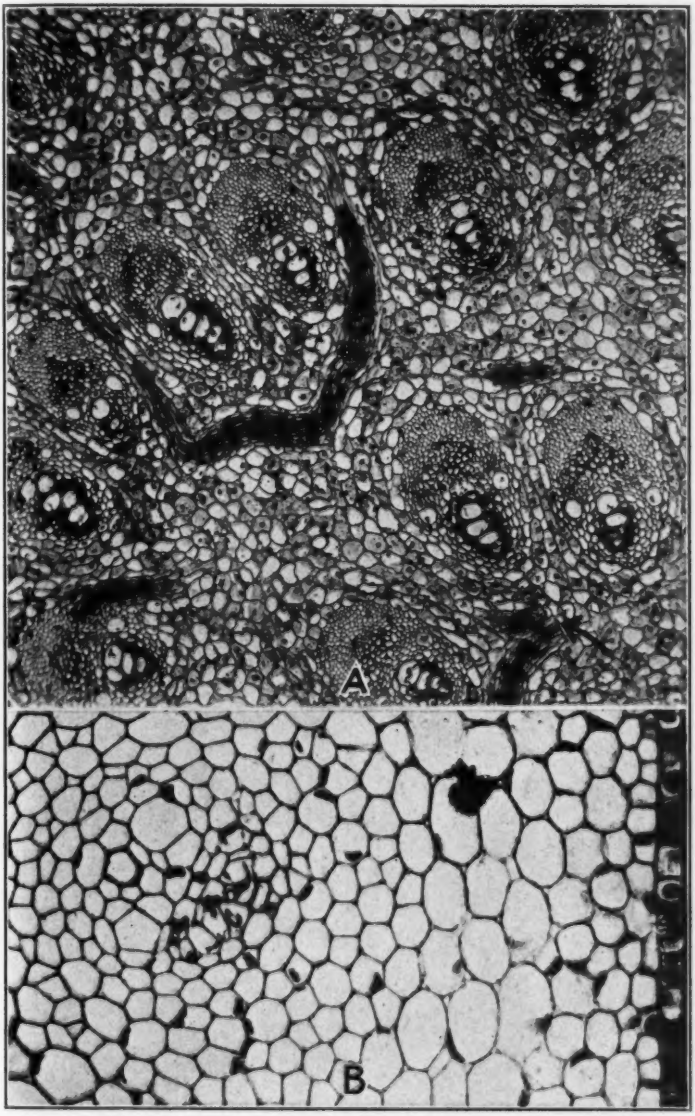


A.—Leaf trace cross section as it appears in its longitudinal course through the node prior to passing out into a leaf. × 200
B.—Cross section of one of the numerous horizontal bundles of the node. There is only one xylem element, but a great deal of phloem. × 510



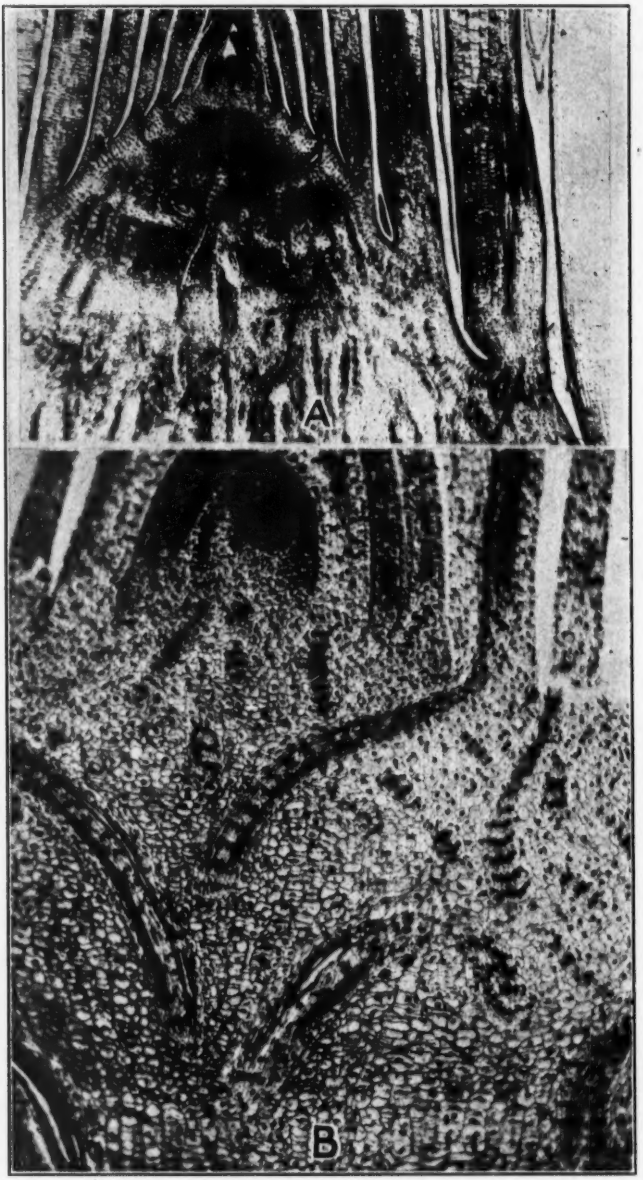
Cross section of large leaf trace as it appears in its longitudinal course through a node near the apex of the stem. There is a great deal of protoxylem and a few scattered scalariform elements at the flanges of the bundle. The bundle is surrounded by parenchyma cells which have a dark granular deposit of tannin and other substances. × 198

A.—Phloem
B.—Scalariform xylem elements
C.—Protoxylem

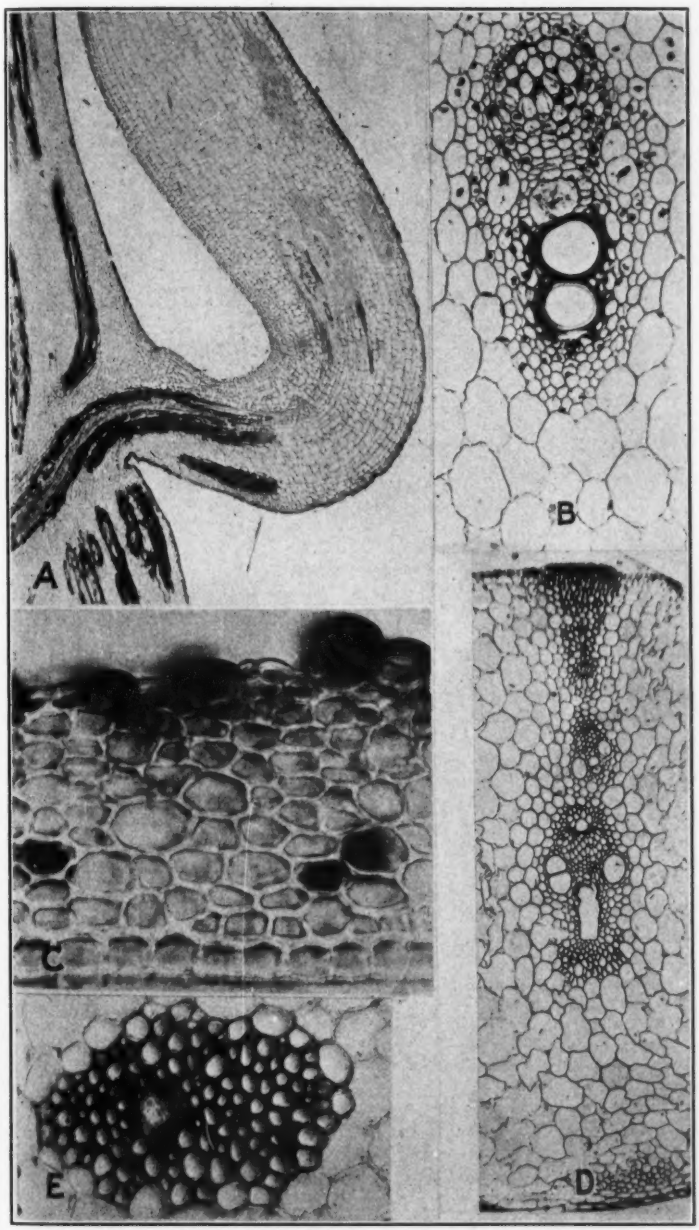


A.—Cross section through the central part of a young node. The vascular bundles are elongated radially. There is a great deal of protoxylem. The section shows also some of the smaller bundles cut longitudinally (designated by the arrow). × 76

B.—Section through a young internode showing epidermis, cortex, and vascular bundles in the state of differentiation. × 468



A.—Longitudinal section through the growing point of a large stem. × 35
B.—Longitudinal section through the growing point showing the curved path of the leaf traces. Notice the crossing of the traces. × 83



A —Longitudinal section of sheath joint to show the passing out of a large leaf trace. × 19
B —Cross section through a young bundle of leaf sheath. Between xylem and phloem is a cambium-like tissue. × 188
C —Cross section through ligule (hand section of fresh material). The dark circles above are hairs cut across. × 480
D —Cross section through a young leaf sheath. For detail see Figure 4. × 66
E —Cross section through a collenchyma bundle inclosing a few phloem cells. × 188

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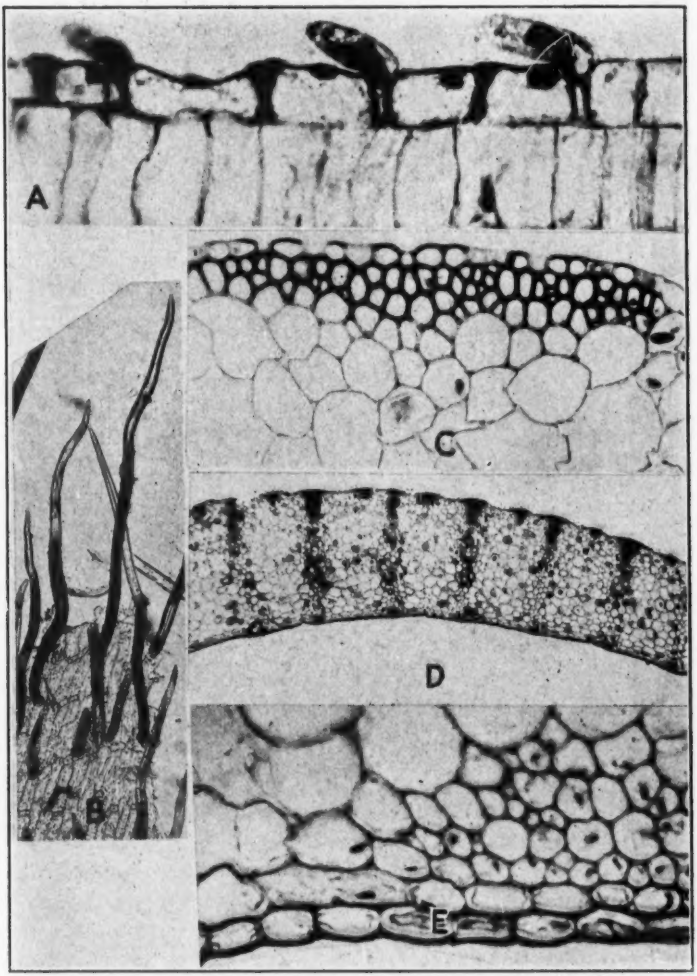
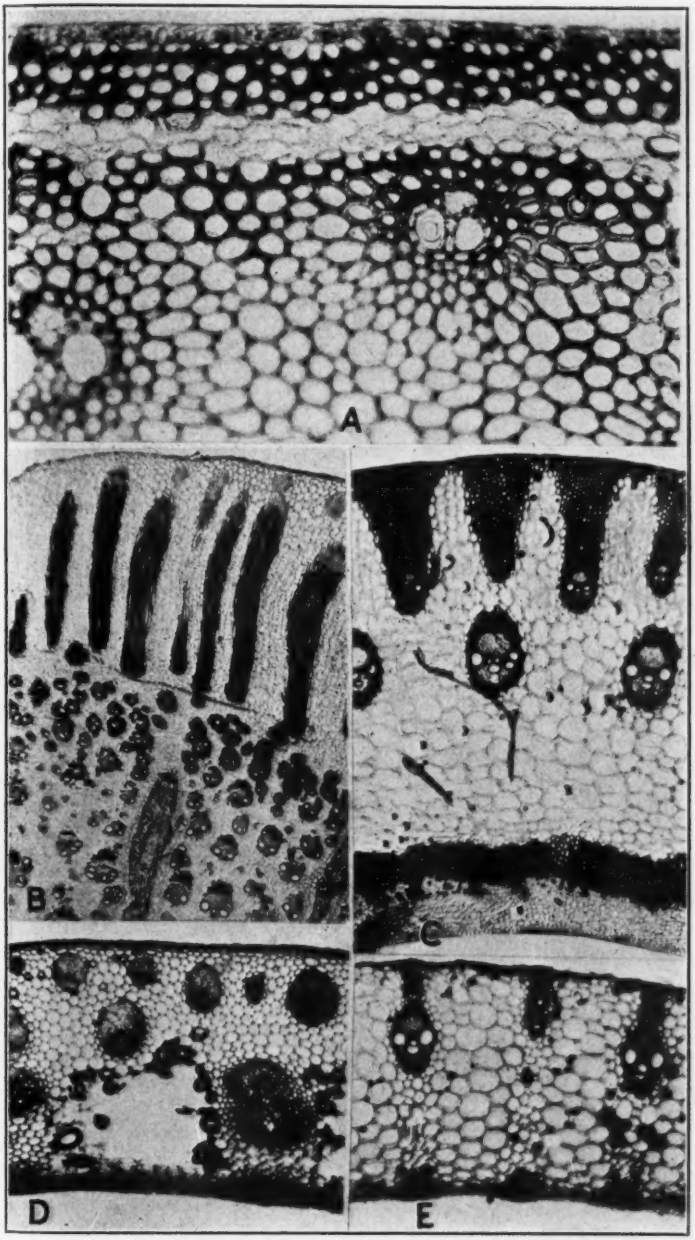
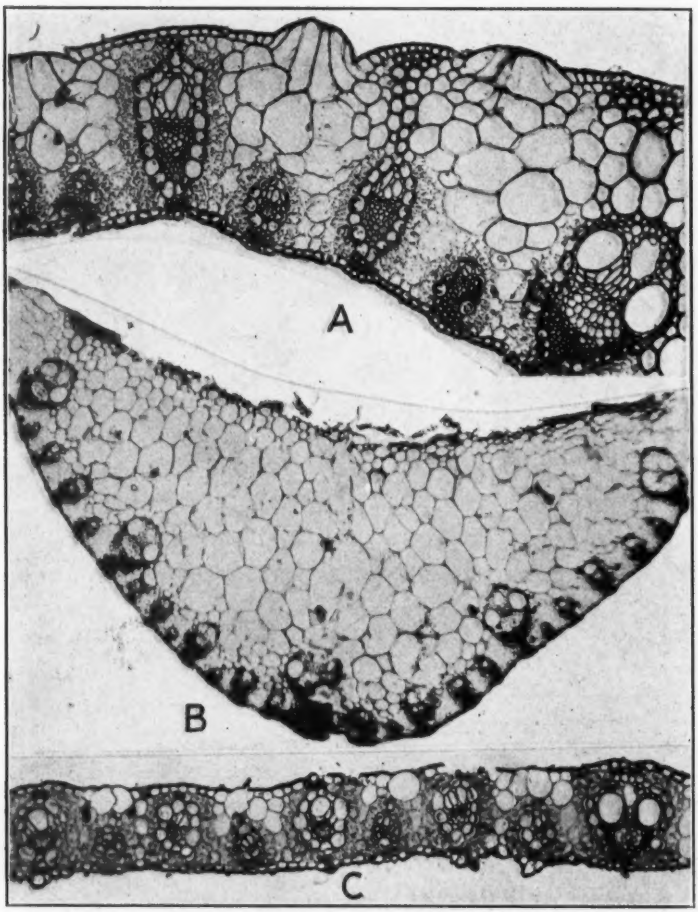


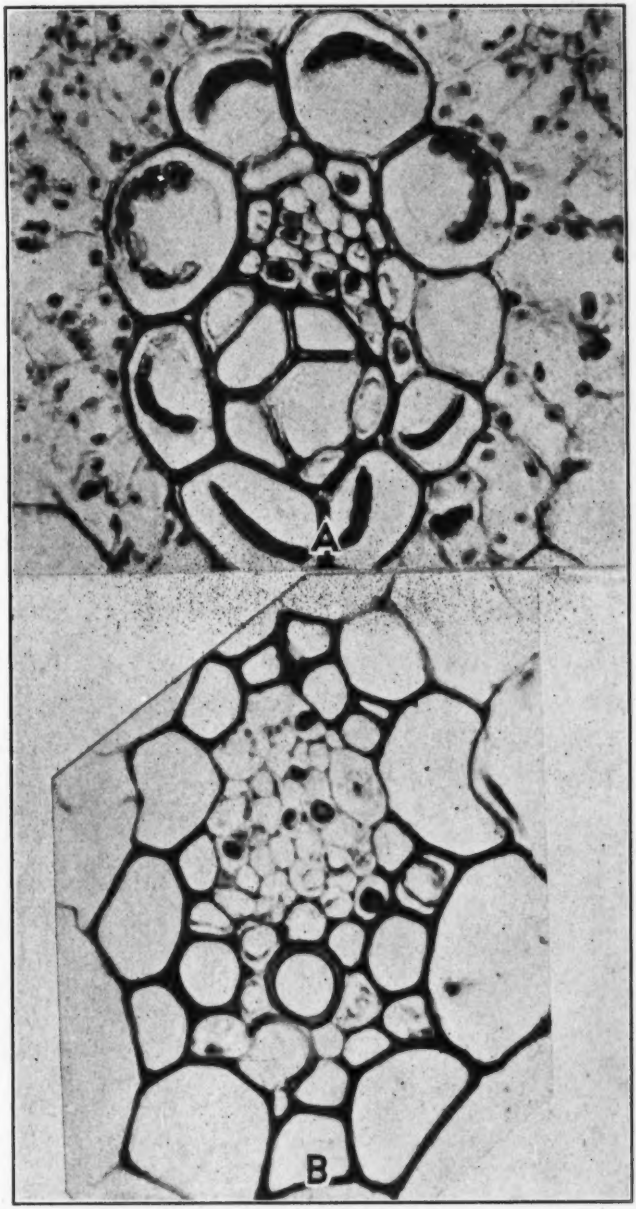
A.—Cross section through a young leaf showing development of hairs. × 468
B.—Epidermis and hairs from sheath joint. × 104
C.—Cross section through young leaf sheath. The epidermis shows both long and short cells; the latter are silicified and appear white in the picture. Beneath the epidermis is a group of lignified sclerenchyma cells. × 412
D.—Cross section through young leaf sheath showing general arrangement of the tissues. × 16
E.—Enlarged view of lower epidermis of the leaf sheath. Notice that the epidermal cells are larger than those shown in C, that the cells of the sclerenchyma are also larger, not lignified and separated from the epidermis by a layer of parenchyma cells. × 321



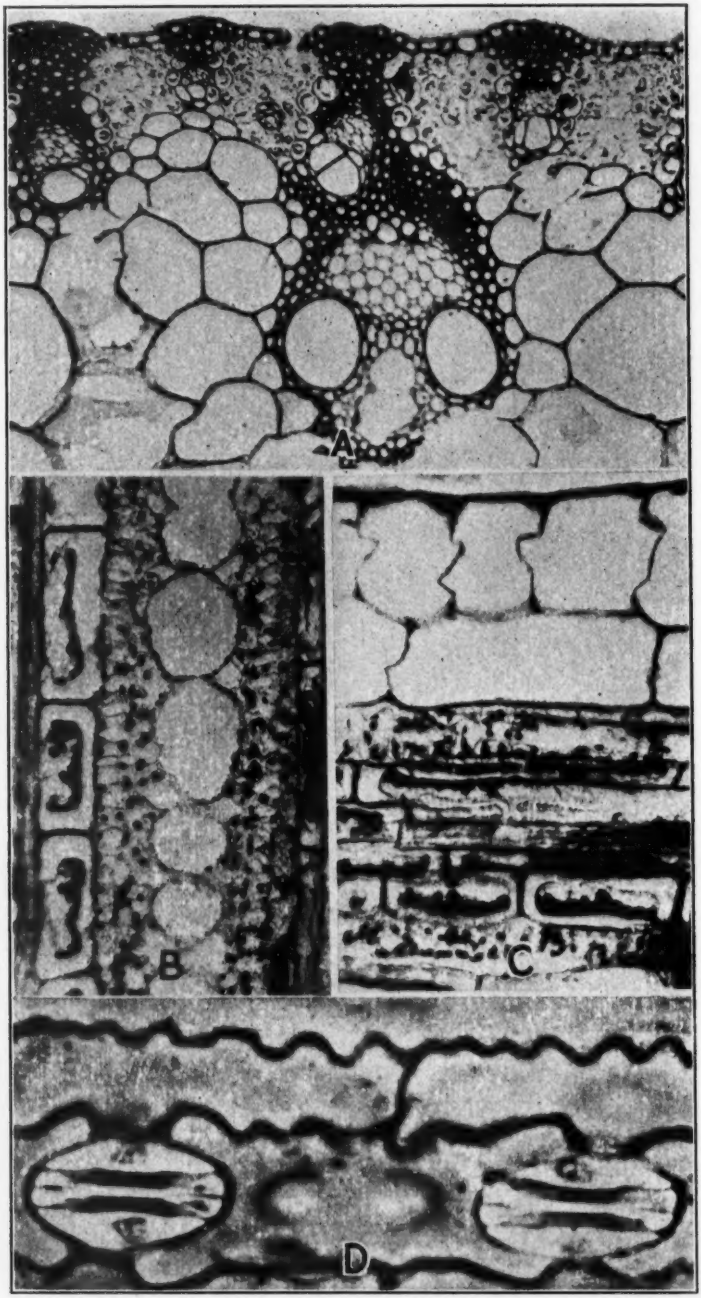
A.—Cross section through peripheral region of mature internode. Beneath the epidermis are several layers of lignified cells, and beneath these are two or three layers of thin-walled parenchyma. For a longitudinal section through this region see text figure 2. ×188.
B.—Cross section through outer stem and leafsheath at the point of the insertion of the latter on the stem. ×19.
C.—Cross section of the sheath at the insertion point of the ligule. The section appears broad since it is taken through the central part of the sheath and not the flanges. ×24.
D.—Cross section through the sheath-joint. ×24.
E.—Cross section through leafsheath midway between the sheath-joint and the blade joint. ×24.



A.—Section through leaf blade in the region near the midrib. × 106
B.—Section through the midrib. × 34
C.—Section through a mature leaf blade. × 88



A.—Cross section through a small vascular group of a leaf. × 890
B.—Cross section through a small vascular group of the leaf sheath. The large-celled sheath around the bundle corresponds to the sheath in Figure A, but the chloroplasts are wanting. The phloem is well developed, the xylem consists of few spiral elements; a protoxylem lacuna is wanting. × 970

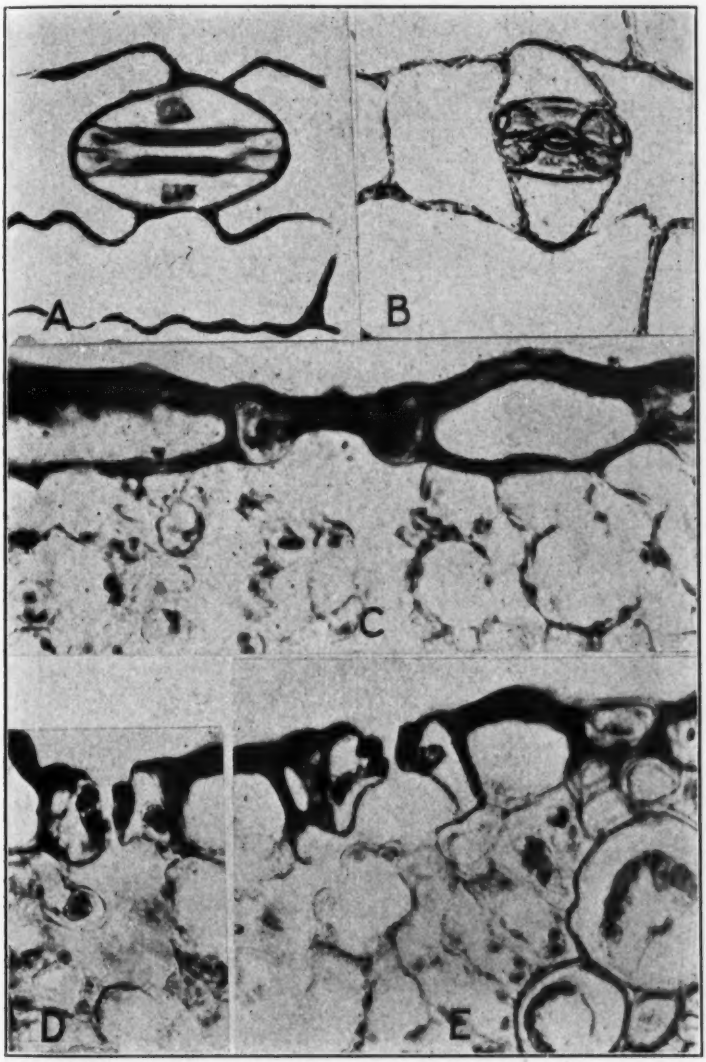


A.—Cross section through the lower surface of mature midrib. The sclerenchyma of the large bundles is continuous with the lignified epidermis. ×165.

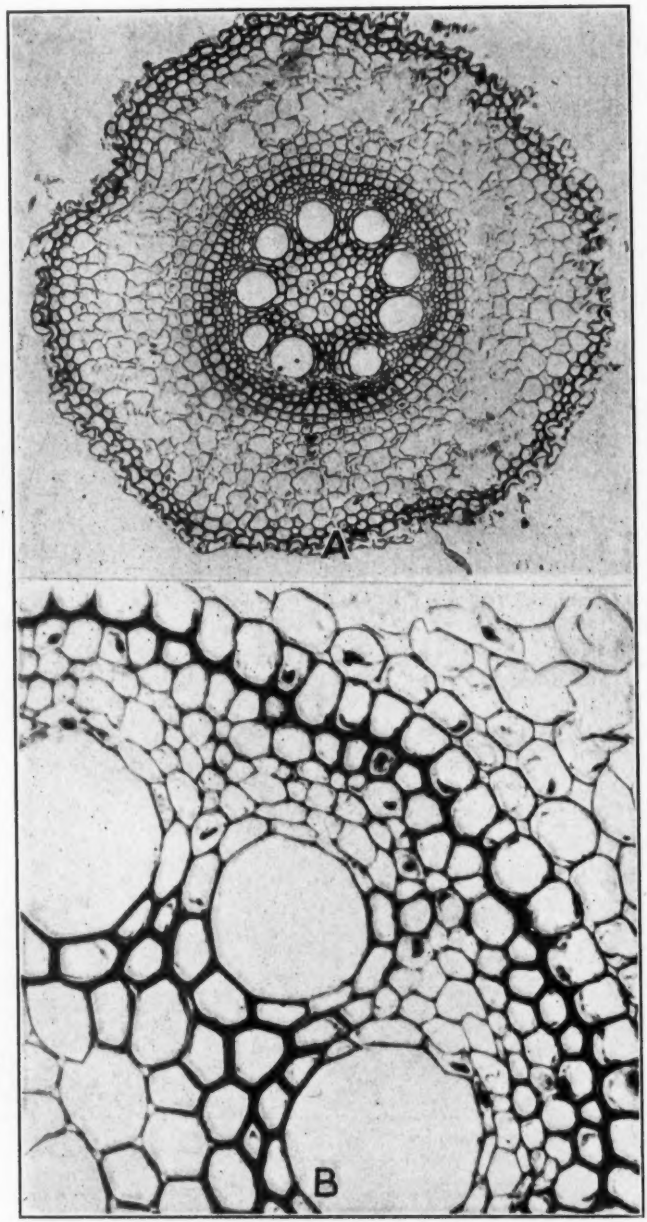
B.—Tangential section through a mature leaf. From left to right: Sclerenchyma fiber, chlorophyll-bearing bundle sheath, spongy parenchyma, bulliform cells, spongy parenchyma. ×340

C.—Radial section through a mature leaf. The upper epidermis in this section is composed of bulliform cells ×340.

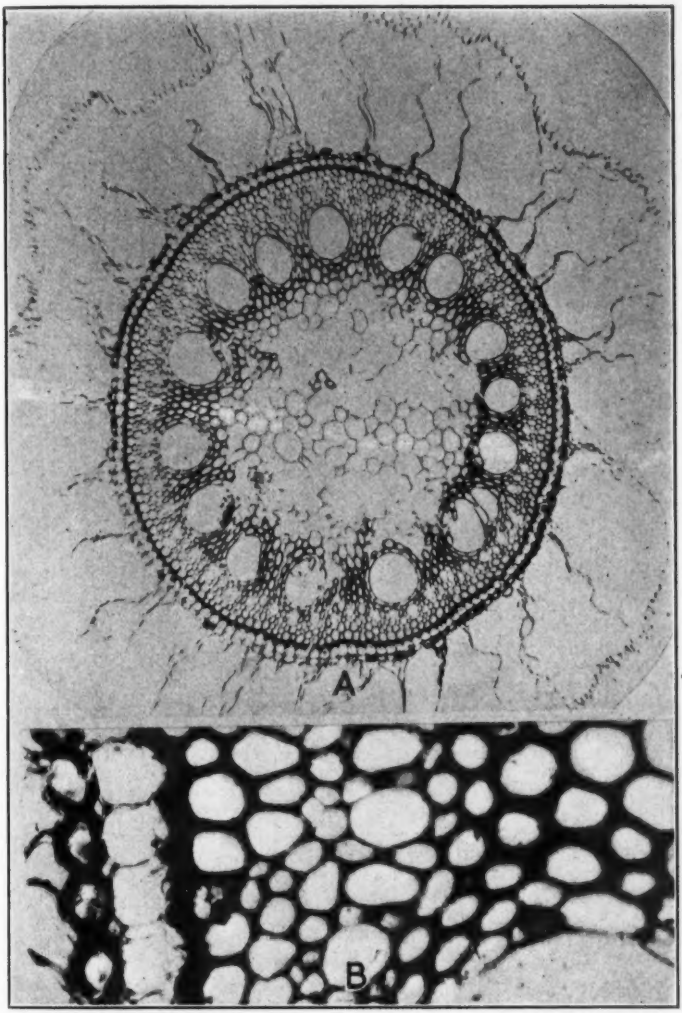
D.—Surface view of epidermis of leaf-bearing stomates. ×816.



A.—Surface view of stomate of a leaf. × 840
B.—Surface view of stomate of inner epidermis of leaf sheath. × 480
C.—Longitudinal section through a stomate of a leaf. × 840
D.—Cross section through a stomate of a leaf. × 840
E.—Cross section through a stomate of a leaf. The second epidermal cell to the right is a short, silicified cell. × 840



A.—Cross section of a fairly young root. × 113
B.—Partial enlargement of Figure A, to show details in structure of endodermis and vascular tissue. × 510



A.—Cross section of a large root. × 76
B.—Enlarged view of A. The row of cells at the extreme left has thickened and suberized walls. Lumen is often filled with gum. × 432

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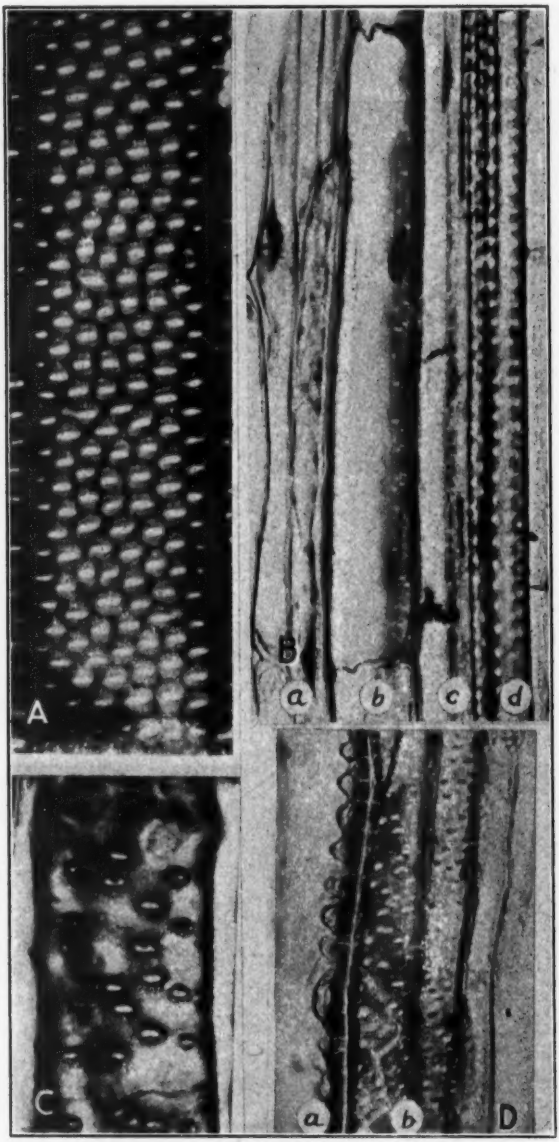
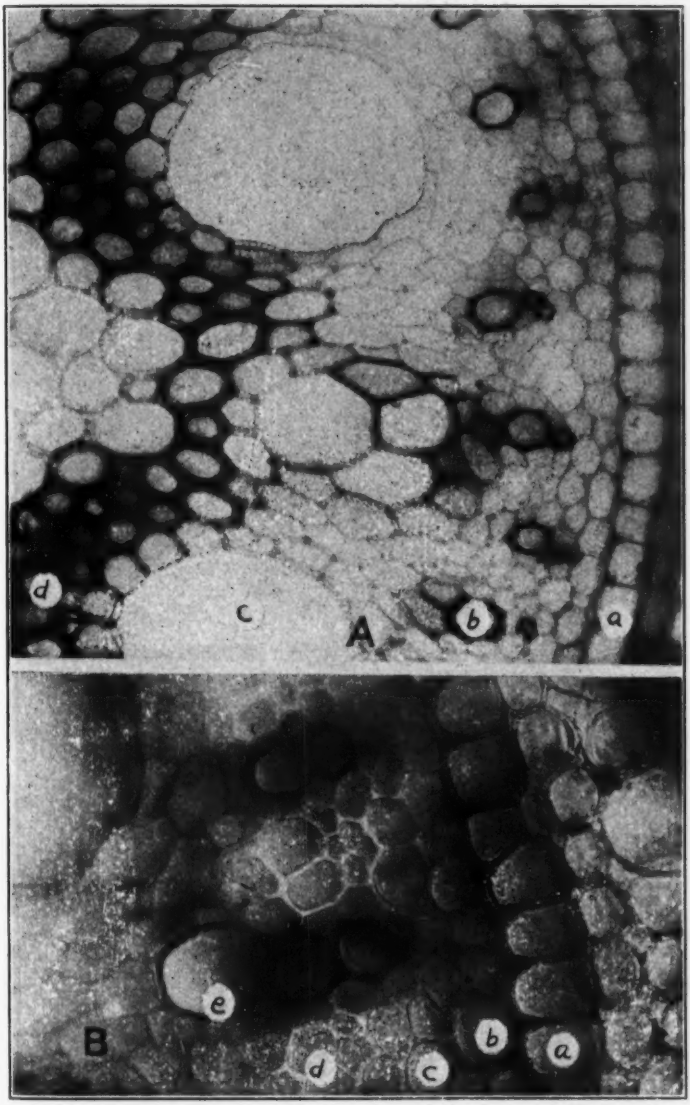
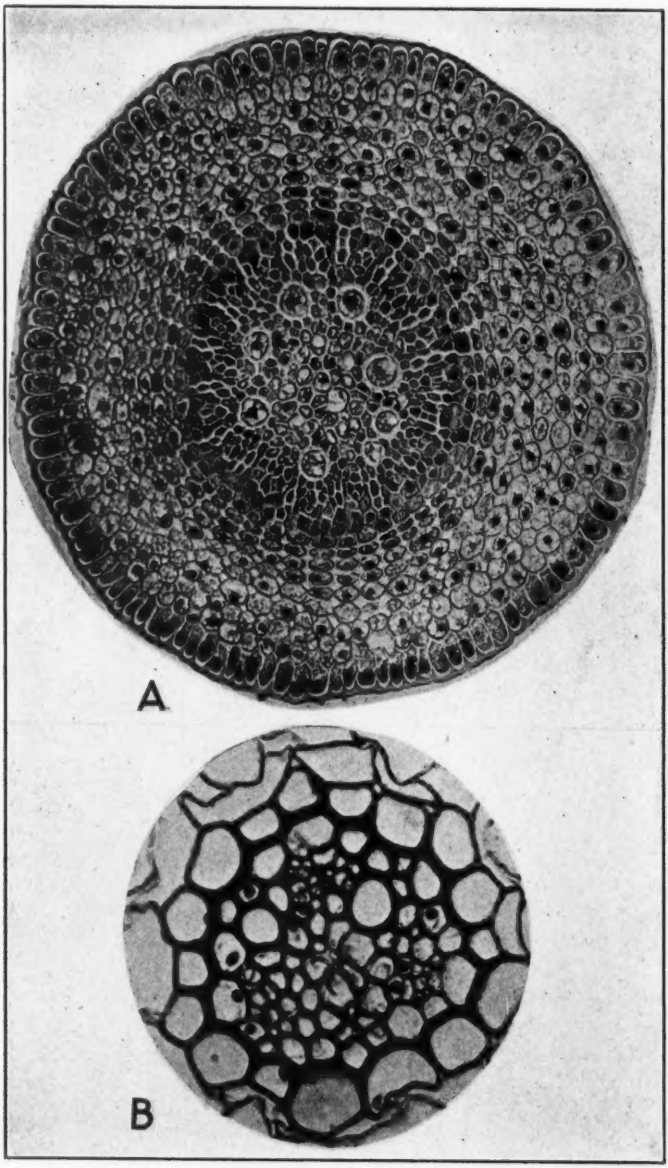


A.—Longitudinal section of large pitted vessel of root. × 1040
B.—Longitudinal section through vascular tissue of root. × 510. a, Inner cortex; b, endodermis; c, pericycle; d, protoxylem
C.—Longitudinal section of endodermis. × 1040
D.—Longitudinal section through vascular tissue of root. × 510. a, Endodermis showing the wartlike protuberances of the tangential wall and the pits between them

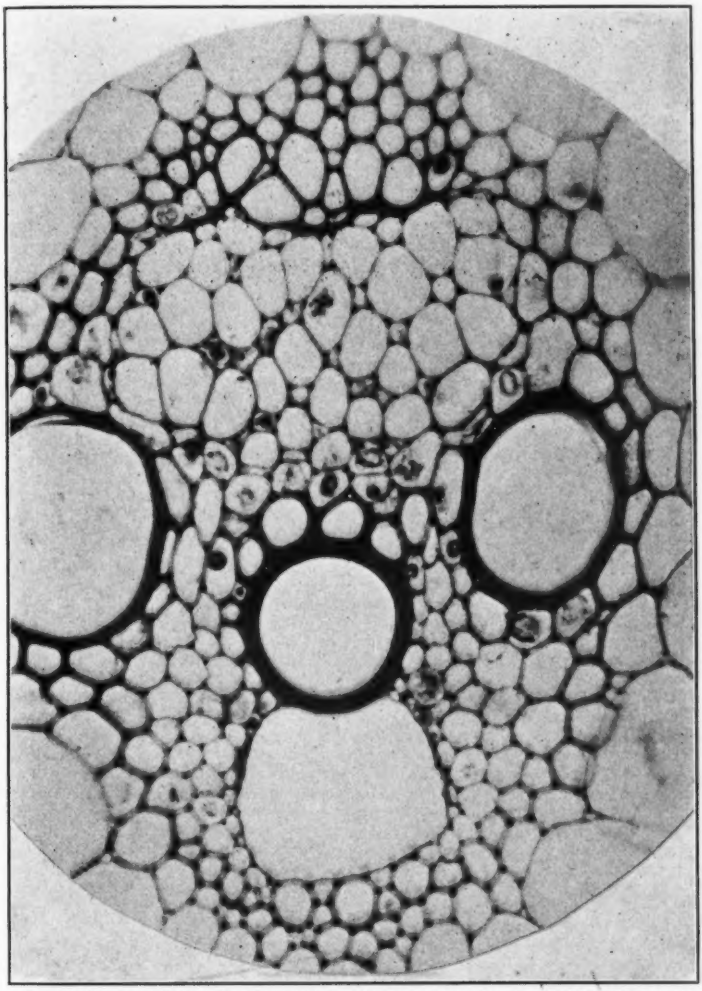


A.—Cross section through the vascular region of a root. × 210. a, Endodermis; b, protoxylem; c, large pitted vessel; d, inner sclerenchyma sheath

B.—Enlarged view of part of Figure A, to show especially the protoxylem and phloem and their relation to each other. × 510. a, Endodermis; b, pericycle; c, interstitial parenchyma; d, phloem; e, protoxylem



A.—Cross section of young root taken slightly above base of root tip. × 200
B.—Cross section of a small lateral root of a seedling. Notice that there are only three protoxylem groups; that a pith is wanting; and that the endodermis is strongly developed. × 510



Cross section through a bundle of a small but mature corn stem. Compared with the bundle of the sugar cane, the former has better developed phloem; more pronounced obliteration of the protophloem and a larger protoxylem lacuna. The cells of the sheath abutting on the protophloem are much larger in comparison with the other sheath cells. × 480

REGARDING THE POSSIBLE ADAPTATION OF SOY BEAN RADICICOLA TO A SPECIFIC HOST VARIETY [1]

By Alfred T. Perkins,[2]

New Jersey Agricultural Experiment Station

INTRODUCTION

It has been noted by several investigators that the number of nodules formed on the roots of soy beans vary in different varieties. J. H. Vorhees [3] reported a case where Haberlandt soy beans failed to produce nodules, even though they were growing with their roots intertwined with those of other varieties which produced nodules. Later experiments have shown that Haberlandt soy beans will produce nodules, but usually to a less extent than other varieties. The question naturally arises as to what this difference in nodulation may be attributed. Theoretically, there are at least two possibilities: First, the bacteria might become adapted, in part at least, to the host variety with which they had been growing in symbiotic relationship; second, the specific host varieties of soy beans might possess different physiological characteristics which would tend to render the several varieties immune to infection in different degrees.

EXPERIMENTAL DATA

Assuming that it is possible for the bacteria to become partially adapted to a specific host variety, the author grew a culture obtained from a single bacterial cell and inoculated Virginia, Haberlandt, Mammoth Yellow, and Wilson soy beans with it. As soon as the inoculations were made, the seeds were planted in fertilized, washed sand free from nodular organisms. When nodules appeared new isolations were made, and the cultures were inoculated back upon the same host variety. Isolations were again made and the cultures which had twice passed through the symbiotic relationship with the same host variety were used in the subsequent tests on the adaptation of the organism to a specific host variety. The tests were conducted in the greenhouse under carefully controlled conditions and in the field where unknown and uncontrollable factors necessarily played a part. The results of the two series of experiments agreed very well.

The tests conducted in the greenhouse were made in flats 2 feet by 2 feet by 4 inches. The flats were filled with washed sand fertilized with magnesium sulphate, monobasic potassium phosphate, calcium carbonate, and ferrous sulphate at the respective rates of 15, 30, 100, and 10 pounds per acre. The inoculations were made by applying the nodular organism directly to the sand. The moisture content of the substratum was maintained by saturating it daily with distilled water. Four flats were used, one for each culture. Each flat was divided into nine sections by stretching strings across the top, and plantings were made with Tokyo, Hollybrook, Mammoth Yellow, Wilson, Virginia, Tar Heel, Haberlandt, Guelph, and Ito San soy beans. After four weeks' growth the crops were harvested and the data on nodulation obtained. These indicate that by twice passing through the symbiotic relationship with a specific host the organism does not become better adapted to that host than to other host varieties. In every case, regardless of the source of the organism, the Mammoth Yellow soy beans produced a greater number of nodules than the other varieties tested. Likewise, Haberlandt and Ito San always produced fewer nodules than the other varieties. These two varieties nodulated approximately to the same degree. In the four cases the relative number of nodules produced by the various hosts was about the same, and no data were obtained which indicated the least adaptation of the nodular organism to a specific host variety. The data secured from the greenhouse experiments are given in Table I.

[1] Received for publication June 11, 1924; issued April, 1925. Paper No. 174 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology. This article is part of a thesis presented to the faculty of Rutgers College in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer wishes to express his appreciation to Dr. J. G. Lipman for helpful suggestions in the planning of this work.

[3] Voorhees, J. H. Variations in soy bean inoculation Jour. Amer. Soc. Agron. 7: 139-140. 1915.

TABLE I.—*Greenhouse experiments showing relative nodulation of different varieties of soy beans when inoculated with organisms isolated from several varieties* [a]

Variety planted	Number of nodules per 10 plants of soy beans when inoculated with organism isolated from—			
	Virginia	Haberlandt	Wilson	Mammoth Yellow
Tokyo	163	182	141	173
Hollybrook	215	185	241	170
Mammoth Yellow	380	421	365	415
Wilson	112	116	170	138
Virginia	98	75	113	122
Tar Heel	150	165	132	161
Haberlandt	53	45	85	63
Guelph	138	142	107	153
Ito San	75	64	51	83

[a] Plants grown for 4 weeks in fertilized washed sand.

The field tests were conducted in cooperation with the State homes at Vineland and Skillman, N. J. Soil that had not been planted in soy beans for years was selected for the plots. Acid phosphate applied at the rate of 250 lbs. per acre was the only fertilizer employed. The inoculations were made by applying the organisms directly to the seeds. The crop was harvested at approximate maturity, the roots of a number of plants being dug in order to obtain the nodulation data. The results of the Vineland experiment are reported in Table II.

The Table II data from Skillman have not been reported, but they are practically identical with those from Vineland. The field tests were limited to 12 plots each. Organisms isolated from Wilson, Haberlandt, and Mammoth Yellow soy beans were used in inoculating these varieties; uninoculated beans were also planted. Since the uninoculated seeds gave none, or at most but few, nodules, they have not been reported on. In the field tests, without exception, Mammoth Yellow produced the greatest number of nodules, Wilson an intermediate number, and Haberlandt the fewest. As in the case of the greenhouse tests, the varieties showed always the same relative ability to nodulate, regardless of the source of the organism. No data were collected which showed a tendency on the part of the organism to become adapted to a specific variety of soy beans.

TABLE II.—*Field experiments showing relative nodulation of several varieties of soy beans when inoculated with organisms isolated from the same varieties.*[a]

Variety planted	Average number of nodules per plant of soy beans when inoculated with organisms isolated from—		
	Haberlandt	Mammoth Yellow	Wilson
Haberlandt	20	18	22
Mammoth Yellow	40	45	50
Wilson	30	26	24

[a] Plants grown to approximate maturity under field conditions.

CONCLUSIONS

The soy bean Radicicola organism does not tend to become adapted to specific host varieties of soy beans.

The differences in nodulation shown by the several varieties of soy beans may be due to some physiological difference in the varieties, possibly a difference in ability to conduct carbohydrates to the roots or proteins away from the roots.

SOWING AND PLANTING SEASON FOR WESTERN YELLOW PINE[1]

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INTRODUCTION

In experiments with the western yellow pine (*Pinus ponderosa*) at the Savenac Nursery at Haugan, Mont., many helpful things have been learned, although it may now be seen that the first years of experimentation were not as effective as they might have been. The problem was attacked in much the same way as that of the western white pine (*Pinus monticola*), because at that time there was no means of knowing that the western yellow pine has no such decided preference for one season of sowing over another as is the case with the western white pine. For at least two seasons sowings were made in the fall and the next spring, with the purpose of watching developments of the following season and comparing the results of the fall and spring sowings. These comparisons were carefully made and they indicated advantages for both sowing seasons. Later, when several sowing dates in the fall were compared, it became evident that the time of sowing within the fall or spring season was a larger factor than season itself. It was realized that efforts should first be directed toward the determination of the optimum time to sow within each season, and that these dates could then be used for comparisons between seasons.[2]

EARLY COMPARISONS OF SPRING AND FALL SOWINGS

Plots of western yellow pine were sown on September 15, 1913, and on May 1, 1914, and observed during 1914. The germination from fall-sown seeds started in the middle of April and was 98 per cent complete before May 18, while the spring-sown seeds had not sprouted a single individual by that date. The plants resulting from the fall sowing were also better developed in most respects than those from the spring sowing. They were slightly more top-heavy, however, the ratio of tops to roots by weight being 4 per cent larger. Weights of 100 and measurements of 200 plants were taken. From these figures it appears that promptness of germination and superior development of the plants are results of fall sowing. (See Table I.)

On October 23, 1915, two beds were sown with 8,000 seeds each, and on May 6, 1916, a similar pair of beds was sown in the same way. The spring-sown beds led in germination by nearly 50 per cent and in survival at the end of the season by 12 per cent. In top-root ratio by weight the fall-sown were more top-heavy by 8 per cent. However, the value of this comparison must be discounted because of the later finding that good results can not be expected from sowing so late in the fall.

The hold-over tendency of seed, so important with western white pine, deserves mention in connection with the western yellow pine. In most cases with fall sowing the hold-over germinations are entirely absent, although 0.2 per cent of hold over has been noted. With spring sowings the tendency is greater. Although it is usually less than 1 per cent, it may amount to more than 25 per cent under certain conditions. The 28 per cent hold over which resulted from a sowing on May 21, 1919, has been attributed to the extremely dry spring that year. (See Table III.)

[1] Received for publication May 22, 1924; Issued April, 1925.

[2] The early field work for these experiments was conducted by E. C. Rogers, assisted by P. C. Kitchin. Their manuscript progress reports cover the earlier experiments cited.

TABLE I.—*Average weights and measurements of 2-year-old seedling stock, western yellow pine*

Date sown	Length of stem	Length of main root	Length of needles	Average number of rootlets, by grades, in each range of root lengths				Weight of top	Weight of root system	Total weight of plant
				Primary		Secondary				
				0.5-2.0 inches	Over 2 inches	0.5-2.0 inches	Over 2 inches			
	Ins.	*Ins.*	*Ins.*	*No.*	*No.*	*No.*	*No.*	*Gm.*	*Gm.*	*Gm.*
Sept. 15	4.0	13.9	2.8	12.8	6.7	1.3	0.05	2.05	0.42	2.47
May 1	3.3	14.1	1.9	12.7	4.4	1.0	.06	1.09	.29	1.38

TABLE II.—*Germination and development of 1-year-old western yellow pine seedlings*

Date of sowing, 1914	Total first season germination	Total loss in seed sown from damping off and sun scorch	Development of seedlings	
			Average length of 200 stems	Average diameter of 200 stems
	Per cent	*Per cent*	*Inches*	*Mm.*
May 1	33.1	3.4	2.89	2.12
May 15	32.3	2.9	2.57	1.98
June 1	26.9	4.8	2.48	1.93

TABLE III.—*Total germination from spring sowings*

Date of sowing[a]	Percentage of seed sown germinating			
	1919	1920	1921	Total
1919				
May 5	10.0	12.7	.....	22.7
May 21	12.5	27.9	.....	40.4
1920				
Apr. 16	.....	62.2	1.9	64.3
May 5	.....	35.9	9.4	45.3

[a] 2,000 seeds sown on each date.

SPRING SOWING

In the spring of 1914 five plots of 2,000 seeds each were sown, one on May 1, two on May 15, and tw on June 1. (See Table II.) Early sowing gave a greater total germination for the first season. This appears to be due to the longer germinating period and to the favorable conditions for germination which occur during May. Early sowing appeared to favor survival slightly, although on this point the figures are not entirely in agreement. At the end of the season larger stock resulted from early sowing. In the spring of 1915 also two sowings of 6,000 seeds each were made on May 6 and May 15. Although these were but nine days apart, the total germination was again larger for the plots sown early and it was also more prompt.

Subsequent to these tests no more sowings were made and no opportunity was afforded to compare different spring sowings until 1919 and 1920, as shown in Table III. The better results in 1920 were again obtained from the earlier spring sowing, though the tendency appears to have been reversed in 1919. The unusual hold over from 1919 sowings has already been mentioned and ascribed to the dry season. The exceptional season may also be the cause of an exception to the general rule of larger totals from earlier sowings.

Both of these tests were observed in connection with a companion series sown on different dates the previous fall. In each case of spring sowing the germination was slow in starting, the bulk of it being in June and July and running through August, while the fall sowings all germinated promptly. The latter were most active in April and May and were complete by the end of June.

Seeds which do not germinate promptly in the spring, as fall and late spring sowings of western yellow pine, result in inferior plants. This has been observed both in sowing at the nursery and in direct seeding trials on planting sites. When germination takes place from the latter part of July to the end of the season, seedlings take on a characteristic abnormal appearance. At the end of the second season in the seed bed they have no fascicles of needles in twos and threes, or only one or two bunches of them. The second season's growth bears pale green straplike leaves, and the bud lacks the strong mature appearance it has in earlier-sowed plants. These inferior plants remain far behind the earlier individuals in development, and tests have shown that when field planted as 2–0 stock [3] they remain at a standstill during the first season. In all shipments of 2–0 stock it is necessary to sort out these plants. In 1915 the loss from this cause was fully 25 per cent of the stock in spring-sown beds. If sowing is done in the spring it should be as early as possible.

FALL SOWING

During 1916 to 1919 sowings were made in the fall on several dates which afford comparisons of the effect on germination of the time of fall sowing. (See Table IV and fig. 1.)

TABLE IV.—*Percentage of germination of western yellow pine seeds sown on various dates in the fall*

DATES OF SOWING [4]

1916

Germination	Aug. 21	Sept. 1	Sept. 12	Sept. 21	Oct. 4	Oct. 14	Oct. 28
Premature (fall)	3.6	4.7	0.0	0.0	0.0	0.0	0.0
Effective (next season)	32.3	30.4	39.2	43.1	50.0	48.5	41.0
Hold over (second season)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	35.9	35.1	39.2	43.1	50.0	48.5	41.0

1917

Germination	Aug. 27	Sept. 7	Sept. 17	Sept. 27	Oct. 6	Oct. 20	Nov. 3
Premature (fall)	7.0	1.0	0.1	0.0	0.0	0.0	0.0
Effective (next season)	33.3	39.4	34.2	38.9	34.6	35.9	20.1
Hold over (second season)	0.0	0.0	0.0	0.0	0.1	0.0	0.2
Total	40.3	40.4	34.3	38.9	34.7	35.9	20.3

1918

Germination	Aug. 27	Sept. 9	Sept. 21	Oct. 1	Oct. 14	Oct. 23	Nov. 4
Premature (fall)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Effective (next season)	69.7	54.5	73.1	67.7	66.8	60.7	54.8
Hold over (second season)	0.0	0.0	0.0	0.0	0.0	0.1	0.5
Total	69.7	54.5	73.1	67.7	66.8	60.8	55.3

1919

Germination	Aug. 28	Sept. 8	Sept. 18	Sept. 28	Oct. 8
Premature (fall)	0.1	0.0	0.0	0.0	0.0
Effective (next season)	54.3	46.8	40.5	46.0	37.8
Hold over (second season)	0.0	0.0	0.0	0.0	0.0
Total	54.4	46.8	40.5	46.0	37.8

[3] In nursery practice the age of plant stock is indicated by figures, the first indicating the number of growing seasons in seed beds; the second, the number in transplant beds. Occasionally a third figure is added to indicate another transplanting. Thus 2-0 indicates 2-year-old seedlings; 1–1–1 indicates 3-year-old stock, 1 year in seed beds and 2 years in transplant beds, but transplanted twice.

[4] 2,000 seeds were sown on each date.

These data for western yellow pine at Savenac Nursery indicate the following conclusions: There is practically no hold-over germination from fall sowings. When sowing is done too early in the fall, considerable "premature" germination, which is lost over winter, may result. It would be better not to sow until after the first week in September. When sowing is done too late, a marked drop in germinative capacity usually results. It would be better not to sow after October 15. Between the two extremes the results show no consistent fluctuations. The five-week period between September 7 and October 15 appears, from our present knowledge, to be a safe time to sow.

results. The effect, however, as has been shown, is to lower germinative capacity and not necessarily to affect the development of plants from the seeds which do germinate. The date of sowing in the spring seems early enough for the average season. The plants used, therefore, may be considered representative of the two classes of stock to be compared.

About April 20, 1918, this planting was duplicated. In each case the necessary precautions were taken to eliminate as far as possible all variables except the one being tested. All planting was done by the same man. The spring and fall planted rows alternated, and plants from spring and fall

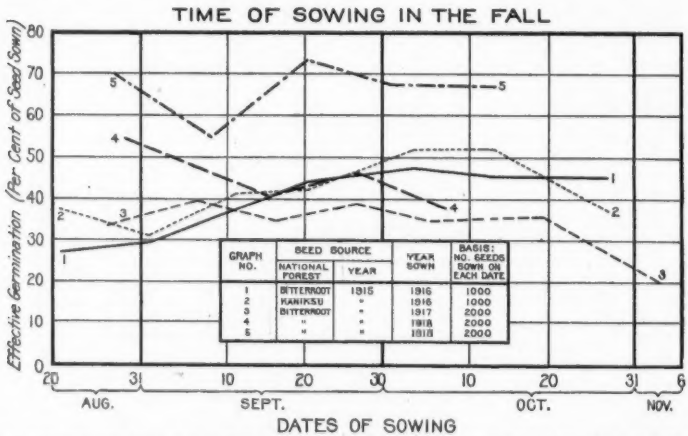

FIG. 1.—Germination of western yellow pine seed at Savenac Nursery as influenced by time of sowing in the fall

It has been observed that 1–2 yellow pine stock from fall sowings is larger, more unwieldy, and more expensive to plant than 1–2 stock from spring sowings. For the production of this class of stock, sowing should be done in the spring.

FIELD-PLANTING EXPERIMENTS WITH STOCK FROM SPRING AND FALL SOWINGS

About October 20, 1917, approximately 600 plants each of 2–0 fall-sown and 2–0 spring-sown stock were planted on a westerly aspect near Savenac Nursery. The fall sowing had been done on October 23 and the spring sowing on May 6 from the same lot of seed. At that time (1915–16) it was not realized that this date in the fall is for the average year about a week late, for best

sown seed alternated within the rows. The plantation was examined five times in 1918 and all plants were grouped in an intensive 12-term classification. The more significant points resulting from this mass of data are reported here.

Six hundred plants from each lot would seem to be an adequate number to yield reliable averages for the test. A smaller number would often be sufficient for quite uniform stock. In order to be sure that all irregularities in the stock were actually compensating within this number, the plantation was divided into four blocks. Each block was examined and recorded separately. The comparisons within each block corresponded closely for all blocks. Therefore in order to simplify this report the tabulations are confined to data from the plantation as a whole.

TABLE V.—*Condition of 2–0 western yellow pine plantations during 1918 expressed in percentage of total number planted (600 each lot each season)*

PLANTED OCTOBER 20, 1917

Condition of the plants	Date of examination									
	June 1		June 17		July 8		July 31		Sept. 20	
	Fall sown	Spring sown	Fall sown	Spring sown	Fall sown	Spring sown	Fall sown	Spring sown	Fall sown	Spring sown
Vigorous	37.7	62.5	38.2	62.5	16.0	34.5	12.5	25.5	14.1	24.6
Failing	23.3	8.0	17.1	7.2	2.9	1.3	1.5	.5	1.2	.5
Dead	9.1	4.0	22.4	12.1	48.9	30.5	68.2	52.4	74.8	58.9
Miscellaneous—unthrifty*	29.9	25.5	22.3	18.2	32.2	33.7	17.8	21.6	9.9	16.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

PLANTED APRIL 20, 1917

Vigorous	91.6	90.8	89.6	90.1	50.9	51.7	37.4	37.3	36.3	32.6
Failing	2.7	1.8	1.5	2.2	.7	.5	.7	0	.3	0
Dead	.7	.7	2.5	2.3	16.0	18.9	46.4	48.2	51.6	53.0
Miscellaneous—unthrifty*	5.0	6.7	6.4	5.4	32.4	28.9	15.5	14.5	11.8	14.4
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

*These percentages cover totals derived from nine additional plants classes which are not reported in detail here because none of them individually show any significant contrast between the behavior of spring and fall sown stock.

At the end of the first season in the field (September 20) following fall planting there were, based on fall sowing, (1) 174 per cent, or three-fourths more vigorous plants in the lot derived from spring sowing; (2) 42 per cent, or less than half as many failing plants among the spring sown; (3) 79 per cent, or four-fifths as many spring-sown plants dead as fall sown; and, on the other hand, (4) 162 per cent, or nearly two-thirds more plants unthrifty in different ways in the spring-sown lot. This last class, however, contains many plants with but slight symptoms of disorder, from which, as is indicated by the 1919 records, most of them recovered. Table V shows that these tendencies were discernible, for the most part, as early as June 1. Injury or death by accident or animal enemies was found to be small and nearly equal in the two lots, hence it does not affect the comparisons.

The second half of Table V shows the results of spring planting as they appear during the first growing season. Taken as a whole, there is very little difference between the lots from spring and fall sown seed. Both, but particularly the fall sown, have done better than when fall planted.

Figure 2 shows graphically the survival in these plantations during 1918. The steeper the curve, the more favorable the water relations, which were evidently at their worst during July. The flattening of the curves at the beginning and end of the season shows the presence of more moisture.

During 1918, also, the current height growth of the season was measured on all thrifty plants. It was as follows:

	Inch
Fall planted, fall sown, 74 plants average	0.66
Fall planted, spring sown, 125 plants average	.83
Spring planted, fall sown, 170 plants average	.76
Spring planted, spring sown, 170 plants average	.80

The spring-sown plants lead the fall sown in both cases, and this lead was greater in the case of fall planting. There was more irregularity in the spring-sown lot, due, undoubtedly, to the variation in germination dates following spring sowing.

In 1919 a simpler plant classification was used for these plantations. (See Table VI.) Again basing percentages upon the results of fall sowing, it may be seen that: (1) 143 per cent, or two-fifths more of the spring-sown than of the fall-sown plants survived when fall planted; (2) 98 per cent, or about equal survival was attained from the two lots when spring planted; (3) 134

per cent,[4] or one-third greater growth for the season was attained by the spring lot when fall planted; and (4) 82 per cent, or four-fifths as great growth was made by the spring-sown lot when spring planted.

Judging from the experiences of 1918–19, then, 2–0 western yellow pine seedlings from spring sowing survive and grow better than those from fall sowing when planted in the fall. When planted in the spring, there is practically no difference in survival as a result of season of sowing, but during the second field season the fall-sown plants lead in growth.

The question naturally arises: Why are fall-sown seedlings of this species less able to withstand the rigors of the planting site when fall planted, and yet fully the equal of spring-sown seedlings when spring planted? This leads to a consideration of the differences between plants from spring and fall sowings. Time of sowing seems to be only an indirect factor, one which is influential only in its effect on time of germination, which in turn affects plant development and hence ability to survive and grow in the field. The inferior plants resulting from late germination were described

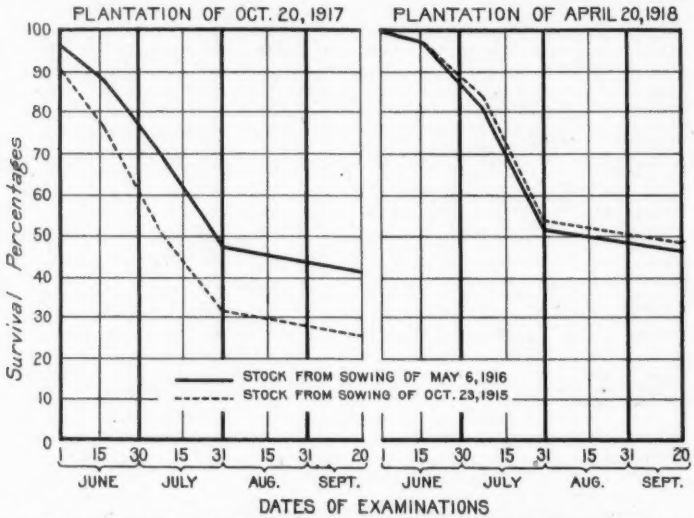

FIG. 2.—First-year survival on the planting site. Fall and spring plantings of western yellow pine 2-0 stock derived from fall and spring sowings (each curve is based on 600 plants)

TABLE VI.—*Records of the second growing season of 2–0 western yellow pine*

Season planted	Season sown	Survival percentages [a]				Growth data	
		Thrifty	Unthrifty	Total living	Total	Average stem-height growth in 1919	Number of measurements [b]
						Inches	
Fall	Spring	13.1	3.2	16.3	83.7	0.82	32
Fall	Fall	7.7	3.7	11.4	88.6	.61	32
Spring	Spring	18.1	4.2	22.3	77.7	.50	94
Spring	Fall	18.2	4.5	22.7	77.3	.61	94

[a] Figures based on examination of 600 plants in each lot.
[b] The largest common number of thrifty individuals used in determining averages.

[4] The basis for this figure is too small to insure accurate averages

under "Spring sowing." The effect of time of sowing upon time of germination has been given, and plant development, as shown in Table I, also has a bearing on the answer to this question. The ratio of tops to roots by weight was 4 per cent larger for fall-sown plants in this case; in other cases it has been found to be 8 per cent larger. The fact that fall-sown plants become more "top-heavy" is believed to be the key to the explanation sought.

When plantings are made in late October, the soil surrounding the roots remains at a temperature [5] low enough to retard water intake by any absorbing surfaces left intact by the planting act. In the meantime transpiration from the tops proceeds, the draft being made chiefly upon the moisture contained in the seedlings when planted. Gradually the saturation deficit is increased until death or a weakened condition, which handicaps the plant the following spring, results. The greater the transpiring surface in proportion to the absorbing surface present and able to function, the faster the drying process proceeds with its injurious or fatal results. Following spring planting, conditions are quite different. New absorbing surfaces are believed to develop after planting, thus preventing any long break in water absorption.

CONCLUSION

The results of these studies show that when western yellow pine is to be field planted in the northern Rocky Mountain region it is much better to use stock resulting from spring sowings for fall planting, but when spring planting is done it makes very little difference whether spring-sown or fall-sown stock be used.

When spring sowing is practiced, it should be as early in the spring as possible.

Fall sowing, if used, should be between September 7 and October 15.

Both seasons of sowing have definite advantages, but, all considered, spring seems to be the safest season as a general policy. Furthermore, spring sowing dovetails well with the general nursery work when there is a large amount of western white pine seed to be sown in the fall.

[5] From Oct. 21 to 27, 1918, at a depth of 5 inches on the site used, the daily maximum soil temperature averaged 42.0° and the daily minimum 38° F.

THE QUANTITATIVE DETERMINATION OF XANTHOPHYLL BY MEANS OF THE SPECTROPHOTOMETER AND THE COLORIMETER [1]

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INTRODUCTION

The quantitative determination of carotin has been discussed in a previous paper.[2] Methods which have been found applicable for the determination of carotin have been found to be equally suitable for the determination of xanthophyll. The greatest difference in the absorption of the two pigments is that the bands in xanthophyll are located farther toward the violet end of the spectrum than are those for the same concentration of carotin. Accurate quantitative data on the spectral transmissive properties of xanthophyll have been obtained by the Bureau of Standards and will be published soon. This paper is concerned only with the spectrophotometric and the colorimetric methods of determining xanthophyll when in solution.

THE SPECTROPHOTOMETRIC METHOD [3]

Since carotin and xanthophyll solutions are very similar as to their light-absorbing qualities, the mercury line 435.8$m\mu$ was used as in the case of carotin. The results given in Table I and in Figure 1 were obtained with different samples of xanthophyll prepared as described below.

In general, the method of preparation of the xanthophyll used in the work described in this paper is the same as that used by Willstätter and Stoll.[4] The complete details of the preparation are to be given with data on the physical properties of the pigment in a later paper. Briefly, the method is as follows: By means of acetone the xanthophyll is extracted from dried cowpea leaves. It is separated from the accompanying carotin and chlorophyll by means of methyl alcohol extractions from a solution of the pigments in petroleum ether. The xanthophyll is extracted from the methyl alcohol by means of ether, which is then evaporated to a few cubic centimeters and methyl alcohol added. The xanthophyll is then recrystallized from methyl alcohol several times. Finally, it is dissolved in chloroform and precipitated from this by the addition of petroleum ether. It is removed from the mother liquor by filtering on a hardened filter, dried in a vacuum desiccator and quickly weighed. A solution of the pigment (0.042 gm. per liter) is made at once and readings taken the same day.

The xanthophyll used to obtain the data in experiment No. 1 and in experiment No. 2 was recrystallized from methyl alcohol and then precipitated from chloroform by the slow addition of petroleum ether. The results in experiment No. 3 were obtained by preparing a sample in the same way as in experiment No. 1 and experiment No. 2. The xanthophyll from which the sample in experiment No. 3 was taken was then dissolved in chloroform and precipitated by the addition of petroleum ether, thus giving the sample for experiment No. 4. The sample in experiment No. 5 was obtained from the xanthophyll of the sample of experiment No. 4 by dissolving it in chloroform and precipitating from petroleum ether. The xanthophyll used in the preparation of the sample for experiment No. 6 was obtained by dissolving the xanthophyll of experiment No. 5 in chloroform and precipitating it therefrom by adding petroleum ether. It was assumed that dissolving the xanthophyll in chloroform and precipitating it by the

[1] Received for publication June 7, 1924; issued April, 1925.

[2] SCHERTZ, F. M. THE QUANTITATIVE DETERMINATION OF CAROTIN BY MEANS OF THE SPECTROPHOTOMETER AND THE COLORIMETER. Jour. Agr. Research 26: 383-400, illus. 1924.

[3] All spectrophotometric data given in this paper were obtained on the König-Martens spectrophotometer at the Bureau of Standards, U. S. Dept. of Commerce.

[4] WILLSTÄTTER, R. M., and STOLL, A. UNTERSUCHUNGEN ÜBER CHLOROPHYLL; METHODEN UND ERGEBNISSE. 424 p., illus. Berlin. 1913.

addition of petroleum ether would give a purer product each time a precipitation was undertaken, but the spectrophotometric data show that this was not the case, for evidently oxidized xanthophyll precipitated along with the pure xanthophyll.

The results for experiment No. 7 were obtained by carefully recrystallizing a sample of xanthophyll five or six times from methyl alcohol and then precipitating it from chloroform by the addition of petroleum ether. The xanthophyll used was obtained from cowpea leaves and was not allowed to stand for any length of time during the purification process. The results in experiment No. 8 were obtained in a similar manner from xanthophyll which had just been extracted from the leaves. Data obtained in experiment No. 9 were gotten by a reprecipitation from chloroform and petroleum ether of the sample used in experiment No. 8.

The xanthophyll of experiment No. 7 and of experiment No. 8 gave the best spectrophotometric results and had a melting point of 174° C. Consequently, an average of the readings for these two samples has been taken as a basis for quantitative spectrophotometric determinations.

TABLE I.—*The transmittancy of xanthophyll in ether* [a]

Experiment No.	Transmittancy [b]	Milligrams of xanthophyll per liter
1	0.0046	6.72
	.0640	3.36
	.0410	1.68
	.4740	.84
2	.0254	4.20
	.0593	3.15
	.1520	2.10
3	.0215	[c]4.20
	.0216	4.20
	.1380	2.10
	.1410	2.10
4	.0232	4.20
5	.0195	4.20
	.0198	4.20
6	.0216	4.20
	.0219	4.20
	.1490	2.10
	.1490	2.10
7	.0180	4.20
	.0181	4.20
	.1350	2.10
8	.0167	4.20
9	.0197	4.20

[a] The xanthophyll was dissolved in ether or absolute alcohol (0.042 g. per liter) and then just before the readings were made was diluted with U. S. P. ether.

[b] For 2-centimeter cell.

[c] Duplicate readings are checks on the same solution.

In determining the purity of a sample of xanthophyll, measurements made on the spectrophotometer are apparently more valuable than melting-point determinations. Melting points were always taken but often these would be apparently correct (173°–174°) and yet the spectrophotometric results would show a difference in the absorption of the different solutions. The solution which gives the greatest light absorption is here considered as the purest. It is quite easy to remove all impurities other than oxidation products from the material. Oxidation products absorb less light than pure xanthophyll, and consequently a product which contains any oxidized xanthophyll would show less light absorption. The graphs in Figure 1 indicate the presence of oxidation products in some of the samples.

In this work the xanthophyll solution (0.42 gm. per liter) which absorbs the most light is considered as being the one which most closely approximates 100 per cent pure xanthophyll, and all of the quantitative data have been based on the graph obtained from the transmittancy of such a solution.

The composite curve of solutions (the average of experiment No. 7 and experiment No. 8) of xanthophyll which gave maximum absorption is plotted in Figure 2 in order to make the data more convenient for use and to avoid confusion with the other experimental data in Figure 1. The manner of using Figure 2 is the same as described in the paper on carotin.[5]

THE COLORIMETRIC METHOD

The colorimetric method of determining the amount of xanthophyll in solutions is similar to that described for carotin. Pure xanthophyll was prepared and 0.042 gram was dissolved in a liter of ether. Colorimetric determinations were made at once on various dilutions of this solution. Three different weighings and determinations (Table II) were made on as many different days, using Lovibond slides and a Duboscq colorimeter.

The graphs in Figure 3, which are the average results of three determinations, show clearly how the colorimeter readings obtained from Lovibond slides 5, 10, and 20 yellow, vary with the concentration of the ether solution of xanthophyll. The curves shown here are used in the quantitative work upon which the interpretation of all

[5] SCHERTZ, F. M. THE QUANTITATIVE DETERMINATION OF CAROTIN BY MEANS OF THE SPECTROPHOTOMETER AND THE COLORIMETER. Jour. Agr. Research 26: 383–400, illus. 1924.

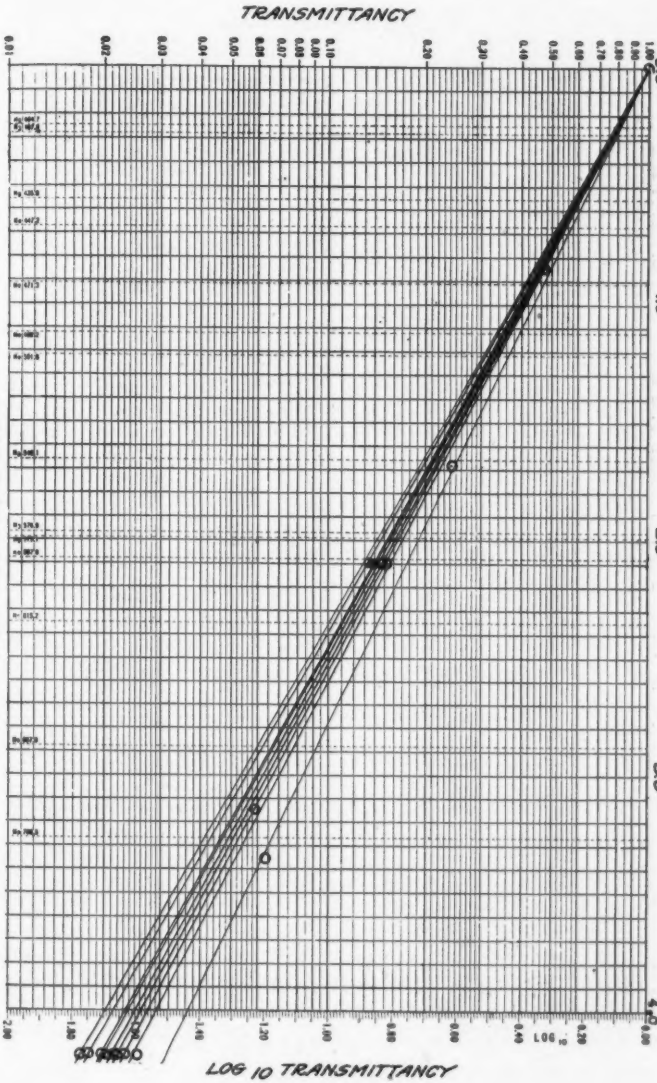

FIG. 1.—Spectrophotometric graphs obtained from ethereal solutions of different preparations of xanthophyll. Prepared from data in Table I

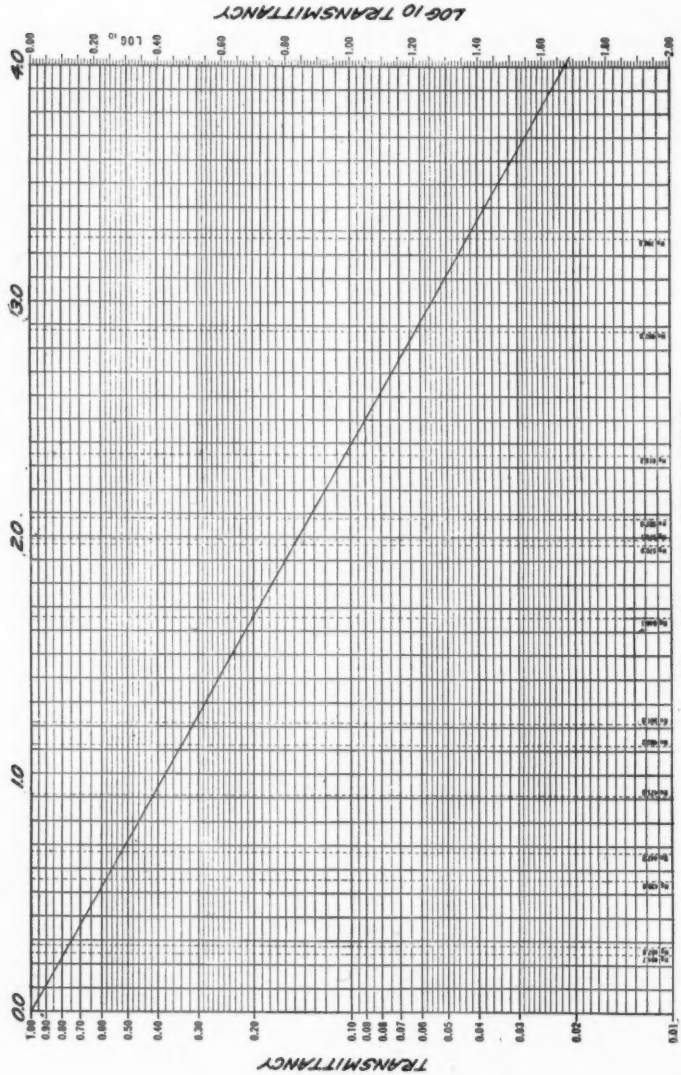

FIG. 2.—Graph of the transmittancy of an ether solution of xanthophyll, using the mercury line 435.8mμ. For use in quantitative determinations

of the colorimetric readings for xanthophyll are based. The chart is used in a manner similar to the chart for carotin in the paper[6] on carotin and consequently needs no explanation.

TABLE II.—*Lovibond slide readings of an ether solution of xanthophyll*

Lovibond slide yellow	Experiment No.	Concentration of xanthophyll in milligrams per liter						
		42.0 mm.	33.6 mm.	25.2 mm.	21.0 mm.	16.8 mm.	8.4 mm.	4.2 mm.
5	1	0.60	0.80	1.1	----	1.5	2.9	6.1
	2	.50	.80	----	1.3	1.6	2.9	5.6
	3	.60	.75	.9	----	1.5	2.9	5.8
10	1	1.00	1.30	1.7	----	2.7	5.2	10.2
	2	1.10	1.30	----	2.00	2.4	5.2	9.7
	3	.90	1.20	1.6	----	2.3	4.8	9.5
20	1	1.60	1.90	2.8	----	4.1	8.0	17.4
	2	1.70	2.10	----	3.3	4.3	8.5	15.9
	3	1.70	2.10	2.9	----	4.1	7.7	15.6
Average	5	.56	.78	1.0	1.30	1.53	2.90	5.83
	10	1.00	1.26	1.65	2.00	2.46	5.06	9.80
	20	1.66	2.03	2.85	3.30	4.16	8.06	16.30

COMPARISON OF SPECTROPHOTOMETRIC AND COLORIMETRIC METHODS FOR THE QUANTITATIVE DETERMINATION OF XANTHOPHYLL

In the former paper on carotin determination it was shown that the spectrophotometer is superior to the colorimeter.

The two methods were compared also in the case of xanthophyll. A solution of xanthophyll in alcohol was used for the test. On September 6 and 7, both clear days, readings (Table III) were taken on the colorimeter each hour of the working day (fig. 4). Similar readings (Table IV) were taken on September 11, using the spectrophotometer. The 2-centimeter cell of the spectrophotometer was refilled for each reading and the solution was diluted 20 times, using ether. The results on the colorimeter, using the solution undiluted, showed a variation of 61 parts in 358, or 17 per cent while the spectrophotometric results, even after the solution used was diluted 20 times, showed a variation of only 10 parts in 354 or 2.8 per cent.

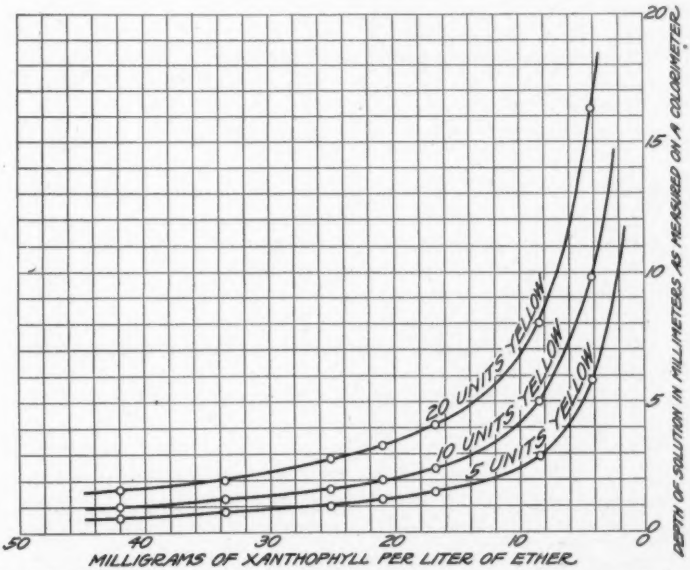

FIG. 3.—Lovibond slide readings plotted to show the results obtained from different concentrations of xanthophyll in ether

The reading in millimeters on the Duboscq colorimeter are plotted on the Y-axis while milligrams of xanthophyll per liter of ether are shown on the X-axis.

[6] SCHERTZ, F. M.—THE QUANTITATIVE DETERMINATION OF CAROTIN BY MEANS OF THE SPECTROPHOTOMETER AND THE COLORIMETER. Jour. Agr. Research 26: 383-400, illus. 1924.

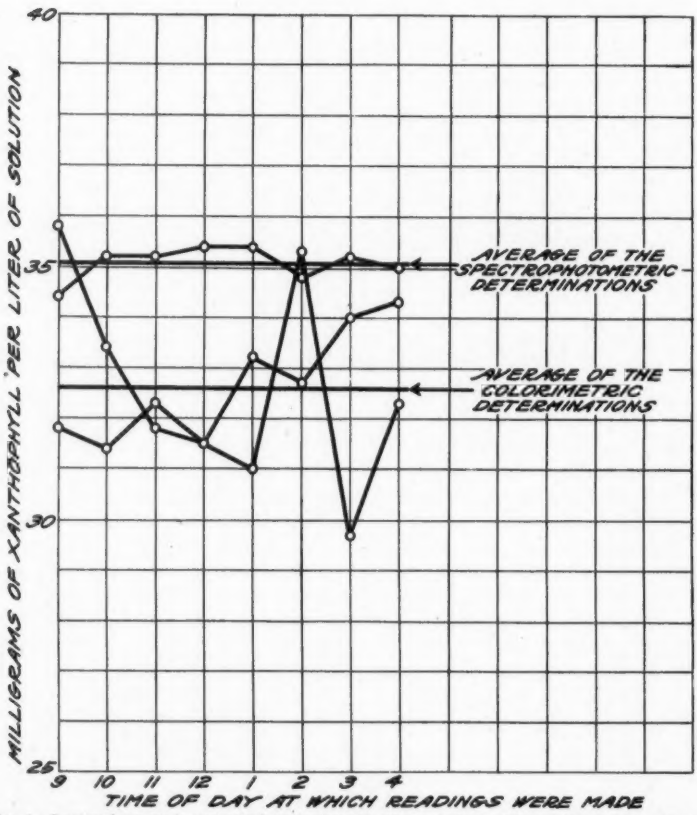
40
35
30
25
MILLIGRAMS OF XANTHOPHYLL PER LITER OF SOLUTION
AVERAGE OF THE SPECTROPHOTOMETRIC DETERMINATIONS
AVERAGE OF THE COLORIMETRIC DETERMINATIONS
9 10 11 12 1 2 3 4
TIME OF DAY AT WHICH READINGS WERE MADE

FIG. 4.—Spectrophotometric and colorimetric determinations, made at different times during the day, plotted to show the variations in the results by the two methods. The upper graph shows the results on the spectrophotometer, while the two lower graphs show the results for the colorimeter. The average for each method is also plotted

TABLE III.—*Colorimetric readings showing determinations made on the same solution at different times of the day*

Date and time of day	Depth in mm. when compared with Lovibond slides			Lovibond slide readings in terms of milligrams of xanthophyll per liter of solution			Milligrams of xanthophyll per liter of solution
	5	10	20	5	10	20	Average
September							
6, 9 a. m.	0.8	1.3	1.7	33.2	33.0	41.2	35.8
7, 9 a. m.	.9	1.3	2.2	30.4	33.0	32.0	31.8
6, 10 a. m.	.9	1.3	1.9	30.4	33.0	36.8	33.4
7, 10 a. m.	.9	1.3	2.3	30.4	33.0	30.8	31.4
6, 11 a. m.	.9	1.3	2.2	30.4	33.0	32.0	31.8
7, 11 a. m.	.8	1.3	2.3	33.2	33.0	30.8	32.3
6, 12 m.	.9	1.4	2.1	30.4	30.6	33.4	31.5
7, 12 m.	.8	1.4	2.3	33.2	30.6	30.8	31.5
6, 1 p. m.	.8	1.3	2.1	33.2	33.0	33.4	33.2
7, 1 p. m.	.9	1.4	2.2	30.4	30.6	32.0	31.0
6, 2 p. m.	.8	1.3	2.2	33.2	33.0	32.0	32.7
7, 2 p. m.	.7	1.2	2.1	37.2	35.4	33.4	35.3
6, 3 p. m.	.8	1.2	2.1	33.2	35.4	33.4	34.0
7, 3 p. m.	1.0	1.3	2.4	26.6	33.0	29.6	29.7
6, 4 p. m.	.8	1.3	1.9	33.2	33.0	36.8	34.3
7, 4 p. m.	.9	1.3	2.1	30.4	33.0	33.4	32.3

TABLE IV.—*Spectrophotometric readings showing the determinations on the same solution as that used in Table III, at different times of the day. (2-centimeter cell and mercury line 435.8 mμ used)*

Time of day	Transmittancy	Dilution	Milligrams of xanthophyll per liter of solution
9 a. m.	0.191	20 x	34.4
10 a. m.	.184	20 x	35.2
11 a. m.	.184	20 x	35.2
12 m.	.182	20 x	35.4
1 p. m.	.182	20 x	35.4
2 p. m.	.186	20 x	34.8
3 p. m.	.184	20 x	35.2
4 p. m.	.185	20 x	35.0

Such results as these obtained for xanthophyll as well as those obtained for carotin reported in a previous paper certainly militate against the use of the colorimeter for accurate work on the quantitative measurement of the plant pigments xanthophyll and carotin.

THE SPECIFIC TRANSMISSIVE INDEX OF XANTHOPHYLL

The specific transmissive index (k) for xanthophyll in ether can be calculated from the graph in Figure 2 by using the equation

$$k = \frac{-\log_{10} t}{bc}$$

TABLE V.—*The specific transmissive index of xanthophyll*

(c) Concentration in centigrams per liter	$-\log_{10}T$ Figure obtained from right-hand side of graph	(b) Thickness of cell	k
		Cm.	
0.400	1.671	2	2.089
.320	1.337	2	2.089
.220	.919	2	2.088
.140	.585	2	2.089
.100	.418	2	2.090
Average			2.089

The average value for K, the specific transmissive index, for xanthophyll in ether is 2.089 for the mercury line 435.8 mμ The specific transmissive index for carotin was found [7] to be 1.986 in ether, using the mercury line 435.8 mμ as the light source.

RELATIVE POSITION OF THE EDGES OF THE ABSORPTION BANDS (I) OF CAROTIN AND XANTHOPHYLL

Since pure pigments were available it was believed that some value might be derived from curves drawn showing the position of the edge, nearer the green, of the absorption band in a Hilger wave-length spectrometer (constant deviation type). Readings (Tables VI and VII), using a narrow slit and a 200-watt lamp as the source of white light, in mμ are plotted on the Y-axis in Figure 5, while the amount of carotin and xanthophyll present per liter is indicated on the X-axis. The quartz glass cells used had widths of 10, 20, and 60 millimeters. The chief value of these curves lies in the fact that they may be used in the identification of an unknown pigment as carotin or as xanthophyll.

[7] SCHERTZ, F. M. THE QUANTITATIVE DETERMINATION OF CAROTIN BY MEANS OF THE SPECTROPHOTOMETER AND THE COLORIMETER. Jour. Agr. Research 26: 383-400, illus. 1924.

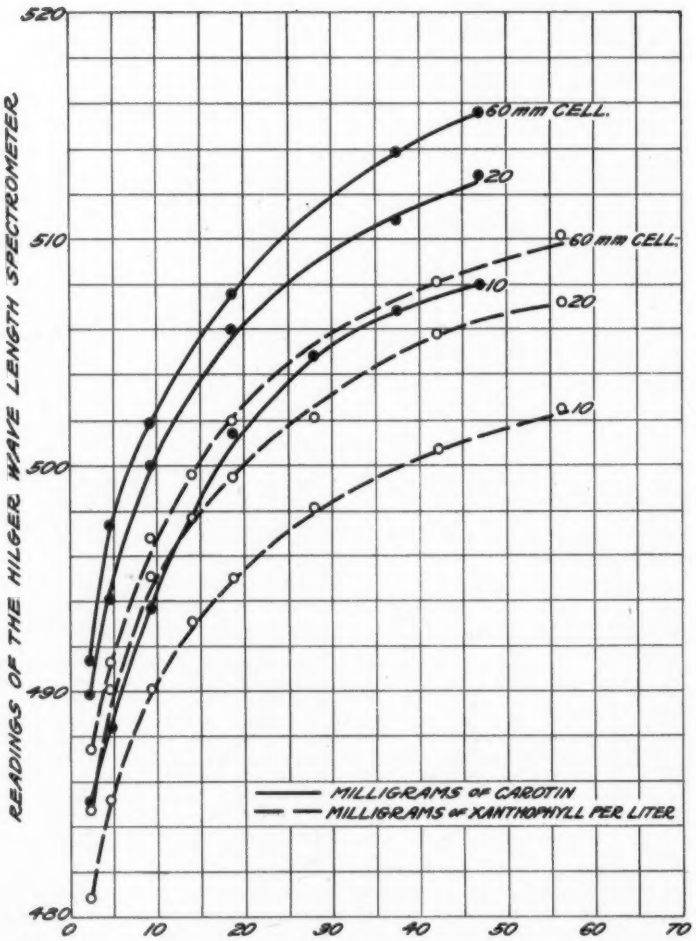

FIG. 5.—Determination made to show the relative position of the edges of the absorption bands (I) of carotin and xanthophyll

TABLE VI.—*Position of the edge of the absorption band (I) of xanthophyll in an ethereal solution*

Thickness of solution	Milligrams of xanthophyll per liter •							
	2.3	4.7	9.4	14.1	18.1	28.2	42.3	56.4
Mm.	mμ	mμ	mμ	mμ	mμ	mμ	mμ	mμ
10	480.9	485.2	490.1	493.1	495.0	498.1	500.7	502.5
20	484.8	490.1	495.1	497.7	499.5	502.1	505.8	507.2
60	487.5	491.3	496.8	499.6	502.0	504.8	508.1	510.1

• Each figure is an average of five or more settings.

TABLE VII.—*Position of the edge of the absorption band (I) of carotin in an ethereal solution*

Thickness of solution	Milligrams of carotin per liter •					
	2.3	4.7	9.4	18.8	37.6	47.0
Mm.	mμ	mμ	mμ	mμ	mμ	mμ
10	485.1	488.4	493.7	501.4	506.8	508.0
20	489.9	494.1	500.0	506.0	510.9	512.8
60	491.4	497.4	501.9	507.6	513.9	515.6

• Each figure is an average of five or more settings.

By testing the behavior of a substance suspected of being either carotin or xanthophyll, toward a mixture of petroleum ether and methyl alcohol [8] and then determining the amount of pigment present per liter by use of the spectrophotometric or colorimetric graphs (figs. 2 and 3), in connection with the graphs (fig. 5) showing where the edge of the band should come, it is easily possible to distinguish carotin from xanthophyll. Solutions of carotin and solutions of xanthophyll of known concentrations were taken and it was found that it was possible to distinguish them by means of Figure 5 alone, that is, by the position of the edge of the absorption bands. It may also be possible to tell whether a certain yellow solution contains carotin or xanthophyll, or neither.

SUMMARY

Graphs are given for various concentrations of xanthophyll in comparison with Lovibond slides 5, 10, and 20, yellow. From these graphs the amount of xanthophyll in a solution may be determined.

The transmittancy of xanthophyll in ether has been determined for the mercury line 435.8 mμ. A graph has been drawn from which it is possible to determine very accurately the amount of xanthophyll in an ether solution.

The specific transmissive index for xanthophyll in ether solution is 2.089, while that for carotin was found to be 1.986. The mercury line 435.8 mμ was used in the determinations.

Data have been submitted which show that the spectrophotometer is accurate to 10 parts in 354 or 2.8 per cent, while the colorimeter is accurate to 61 parts in 358 or 17.0 per cent.

A graph (fig. 5) has been made showing the relative position of the edge of the absorption band (I) for carotin and for xanthophyll. These may be used to ascertain whether a solution contains carotin or xanthophyll, after the concentration has been determined.

[8] Carotin will separate in the petroleum ether layer while xanthophyll will be found in the methyl alcohol layer.

CONTRIBUTION TO THE CHEMISTRY OF DECOMPOSITION OF PROTEINS AND AMINO ACIDS BY VARIOUS GROUPS OF MICROORGANISMS[1]

By SELMAN A. WAKSMAN and S. LOMANITZ

New Jersey Agricultural Experiment Station

INTRODUCTION

Extensive work has been done on the decomposition of proteins and their derivatives by microorganisms, especially by bacteria and fungi. In the great majority of cases one of the final products, ammonia, has been used as a criterion for the course and rapidity of this decomposition; witness only the large number of contributions to the so-called "ammonification" studies, both by crude and by pure cultures of microorganisms, in investigations dealing with soil microbiology. Whenever bacterial metabolism has been studied, especially nitrogenous metabolism, ammonia has usually been the product to be measured, some authorities laying more emphasis upon this as an index of bacterial decomposition of proteins (*12*)[2] and some less (*5*). Very little attention has ordinarily been paid to the transformation of the carbon part of the protein molecule or to the transformation of the available carbon present in the medium, in addition to that of the protein molecule; at least not to such an extent as to indicate the importance which this transformation plays, not only in connection with the formation or accumulation of ammonia, but with the decomposition of the protein molecule.

The purpose of this contribution is to indicate how the structure of the particular amino acids and of various proteins consisting of different amino acid groupings influences the mechanism of their decomposition by representatives of the three different groups of microorganisms, which play an important part in the decomposition of proteins in nature—the bacteria, actinomycetes, and fungi or molds.

LITERATURE

The three methods most commonly used for measuring the rapidity and amount of decomposition of proteins can be summarized as follows:

Disappearance of original protein, as in the liquefaction of gelatin, coagulation and liquefaction of milk casein, zone formation on casein agar plate, etc.—Very little attention has been paid, however, to the actual determination, in an accurate quantitative manner, of the amounts of protein decomposed. Even the numerous gelatin liquefaction studies are more qualitative than quantitative in nature. An approach has been made to quantitative methods only by the introduction of measurements of viscosity in the study of gelatin liquefaction (*30*); but even here, no differentiation has as yet been made between the mere change in the physical condition of the gelatin and its actual chemical hydrolysis, since liquefaction of gelatin may be brought about by enzymes (gelatinases), and this action may be entirely independent of proteolytic activity. Berman and Rettger (*1*) pointed out that the ability of an organism to liquefy gelatin is no sure indication of its proteolytic properties.

Formation of intermediary products in the decomposition of proteins, chiefly amino compounds.—Berman and Rettger (*1*) used the biuret test for following the course of protein hydrolysis. Sears (*25*), Waksman (*33*), and others used the Van Slyke method for determining the amino acid nitrogen as a method for following the course of decomposition of proteins. Itano (*9*), Kendall (*13*), Berman and Rettger (*1*) and others used the Sörensen (*26*) formol titration method; while DeBord (*5*) used the Folin (*8*) method for determining amino nitrogen. The determination of amino nitrogen as an index of decomposition of proteins should be used, however, only when the particular processes carried on by the different organisms are properly understood. When the protein is present as the only source of carbon and nitrogen in the medium and has to be used by the pure or mixed culture, as a source of both nitrogen and energy, the fact that the mere hydrolysis of

[1] Received for publication May 1, 1924; issued April 1925. Paper No. 167 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

[2] Reference is made by number (italic) to "Literature cited," p. 280.

the protein will liberate only a small amount of energy must be considered; the organism has to decompose the amino acids and other nitrogen derivatives of the protein molecule, and different organisms may attack different amino acids with a different degree of rapidity.

The amino compounds are therefore formed and decomposed; they will accumulate in the medium only if the particular organism is unable to use these compounds or the larger part of them, formed from the protein; they will disappear only when the organism attacks them as readily as it does the protein or when another organism is present that attacks these compounds as soon as they are formed by the other organism from the protein molecule. It would be expected that different organisms will not only differ in the rapidity and completeness with which they are able to hydrolyze one protein, and especially different proteins, but also in the kind of amino acids which they are able to utilize as sources of carbon and nitrogen. Taylor (*27*), for example, found that *Bacterium coli* did not produce amino acids from casein, but *Proteus vulgaris* produced lysin and histidin. When an available carbohydrate is present in the medium, the organisms may prefer this as a source of energy and attack the protein only as a source of nitrogen; they may, however, prefer the protein to the carbohydrate also as a source of energy. This is the reason for the various discrepancies reported in the literature on the sparing action of carbohydrates. When the carbohydrate is utilized in preference to the protein as a source of energy the latter is decomposed only to a limited extent, according to the nitrogen requirements of the organism; therefore the ammonia and perhaps even the amino nitrogen may not accumulate and may even diminish. When the protein is preferred as a source of carbon to the available carbohydrate, the amino nitrogen and ammonia may accumulate.

There is another possibility when an organism prefers only certain amino acids as sources of nitrogen, that it will decompose large quantities of protein to enable it to obtain the particular amino acids, leaving the others to accumulate in the medium. Thus the amount of amino nitrogen formed from the decomposition of proteins may fluctuate, indicating that it is continuously formed and decomposed by the organisms, as shown by Sears (*25*); or steadily increasing concentrations may be obtained for strong proteolytic organisms, as in the case of *Bacillus subtilis* and *B. pyocyaneus*. Waksman (*33*) found that the *bacterium B. coli* gave an increase of amino nitrogen in plain broth while the fungus *Aspergillus niger* showed a steady decrease. DeBord (*5*) found that the presence of glucose in peptone media increases the rate of production of amino nitrogen in cultures of various bacteria.

Use of ammonia as an index of decomposition of proteins and their derivatives by microorganisms.—One advantage of this method consists in the fact that ammonia is the end product of protein transformation and as such it is not transformed further by heterotrophic microorganisms unless there is an available nonnitrogenous substance present as a source of energy. The great advantage of this method is that the largest part of the ammonia accumulates in the medium not as a result of the nitrogen metabolism of the organism, but as a result of its carbon metabolism, when proteins and amino acids are used by microorganisms as sources of carbon, both as a source of energy and for structural purposes; ammonia is then left as a waste product of respiration of the microorganisms. This is true for both bacteria and fungi and has been well recognized by the early bacteriologists, such as Marchal (*17*). The process of ammonia accumulation has been used most extensively in the literature of microbiology as an index of protein decomposition, especially so in the branch of the subject applied to soils, because of the difficulty of extracting and determining residual proteins or intermediary products in the soil as a medium, and the ease of ammonia determination. The term "ammonification" has come into general use, although the term "ammonization" would be the more proper one, since it came first into use in the nineties of last century, when suggested by Marchal.

According to Butkevitch (*3*), ammonia formation is not the final stage in respiration, but the first stage, since ammonia is first split off (deaminized) from the amino acid, leaving the remaining group available as a source of energy for the organism.

Ammonia accumulation can serve as a good index of proteolysis only when no available carbohydrates are present; in the presence of the latter this method should be supplemented by the study of another process, either the disappearance of original protein, or the formation of amino nitrogen. This accounts for the fact that Kendall and Walker (*12*), Butkevitsch (*3*), Marchal

(*17*) and numerous other investigators found ammonia formation a good index of proteolysis, while DeBord (*5*) and others found that it is not a reliable index. The term is merely relative and should not be made to mean any more than it does.

When a protein is acted upon by microorganisms it is first hydrolyzed into various protein derivatives, including the amino acids; the latter are then deaminized. It is important to know whether all constituents of the protein molecule are acted upon alike by microorganisms, or whether some are acted upon first and others after. Robinson and Tartar (*22*) found that all the nitrogenous compounds are changed more or less by the bacteria with the formation of ammonia; in no case was one of the derivatives completely destroyed, but the rapidity of action varies with the different proteins. Lathrop (*15*) pointed out that the monoamino and diamino acid nitrogen (especially the latter, according to Kelley (*11*)) are the chief sources, in the protein molecule, from which ammonia is formed.

There are available, however, undisputed facts in the literature that certain organisms prefer one chemical group to another and that different organisms may vary in their preferences in this respect. Rubner (*23, 24*) demonstrated calorimetrically that bacteria consume a great deal more material for energy than for growth (increase in protoplasm). *B. proteus*, for example, utilized, in a period of 10 days, 3.01 calories for growth, out of 12.32 calories transformed; longer periods of growth even increased the ratio of the number of calories used for energy to that used for growth. Growth and energy consumption are so related that they are affected alike by environmental conditions, as temperature. Different species, however, were found by Rubner to behave differently in this respect.

The fungi producing an abundant growth and assimilating a large amount of the available carbon behave differently in this respect; about half of the nutrient consumed is used for resynthesizing the cells (*14*); Terroine and Wurmser (*28, 29*) found that *Aspergillus niger* stores in the mycelium as much as 59.6 per cent of the metabolizable energy. It may thus be concluded that, as a general rule, bacteria utilize a much smaller amount of energy than fungi for growth and the synthesis of protoplasm.

As to the decomposition of different amino acids and acid amides in the soil, which contains a mixture of a large number of organisms, Jodidi (*10*) demonstrated that the rate of their transformation into ammonia is greatly influenced by their chemical structure; amino acids and acid amides of equal structure were found to yield about the same proportion of ammonia.

According to Blanchetière (*2*), alanine, leucine, aspargine, phenylalanine, tyrosine, and histidine are readily utilized by *Bact. fluorescens liquefaciens*, as sources of both carbon and nitrogen; glutamic acid and tryptophane are utilized more slowly, and glycocoll only after a period of incubation, longer than one month.

EXPERIMENTAL DATA

A standard medium, synthetic in composition, has been used in the following investigations; it contains the following mineral constituents:

K_2HPO_4	1.0 gram
$MgSO_4 .7H_2O$	0.5 gram
NaCl	0.1 gram
$FeSO_4$	0.02 gram
Distilled water	1,000 c. c.

One per cent of the various proteins and amino acids was added to the above medium. Pure or anhydrous dextrose was used in 2 or 1 per cent concentrations. The amino acids were purified commercial products and consisted, therefore, of the racemized forms. The casein was purified according to Hammarsten and brought into solution, by dissolving 1 gm. of casein in 8 c. c. of 0.1N NaOH then adjusting the reaction with HCl. The media were placed, in 100 c. c. portions, in 250 c. c. Erlenmeyer flasks, plugged with cotton and sterilized at 10 pounds for 30 minutes. The media were then inoculated with the various organisms grown on agar slants.

The following organisms have been used in this study:

Trichoderma koningi Oud. and *Zygorhynchus mölleri* Vuill. isolated from the soil and kept in culture for some time.

Actinomyces viridochromogenus (Krainsky) Waksman isolated from the soil in 1916 and described in detail elsewhere.

Bacillus cereus Frankland isolated from the soil.

Bacterium fluorescens (Flügge) Lehm. et Neum. isolated from the soil.

The following procedure of analysis was used. At the end of the period of incubation the cultures were filtered through weighed pieces of ashless filter paper and the mycelium washed with a little distilled water so as to bring the filtrate to original volume. The mycelium was then dried to constant weight at 65° to 85° C., then analyzed for

total nitrogen by the Kjeldahl method. The ammonia nitrogen was determined in an aliquot portion (25 c. c.) by the Folin (*7*) aeration method, using 5 c. c. of 40 per cent Na_2CO_3 solution and some heavy oil, to prevent foaming, and aerating, for 4 hours, into $\frac{N}{10}H_2SO_4$, then titrating. The amino nitrogen of the liquid was determined by the Van Slyke micro method (*31*, *32*), after the ammonia had been aerated, or, in the original solution, allowing for the ammonia content by subtracting one-half of the ammonia nitrogen from the total amino nitrogen (shaking 5 minutes). The total nitrogen in the solution was obtained by determining it in an aliquot portion of the filtered culture. The residual dextrose was determined by the Bertrand method. The hydrogen ion concentration of the medium was determined by the colorimetric method. In view of the fact that, in the case of the bacteria, the mere filtration through paper gave the weight of the bacterial pellicle but not of the cells throughout the medium, attempts have been made to obtain the amount of the latter by carefully acidifying with acetic acid, then filtering the coagulated mass. This process has been carried out successfully only in the case of the bacteria growing on glutamic acid media. The total weight of the bacterial cells, in the other culture, is therefore only approximate and indicates merely the weight of the pellicle. In the case of the fungi and the Actinomyces, however, filtration through paper is sufficient to separate the growth from the culture fluid completely.

In all cases duplicate cultures were prepared, using an uninoculated control for every fresh lot of medium; only the averages of the two determinations are recorded; in those cases where the results in the duplicates varied markedly they were reported separately. Several of the experiments were repeated, but unless markedly different results were obtained (as in the case of the 1.4 per cent glutamic acid) they were not recorded.

Before discussing the various chemical changes produced by the different microorganisms it may not be out of place to point out several outstanding characteristics in the growth of these organisms. The *Bact. fluorescens* used the carbon and especially the nitrogen of the various amino acids in a most excellent way, producing a heavy pellicle on the surface, especially so in the presence of dextrose; the characteristic fluorescent pigment was produced in most cases on the second or third day of growth only in the amino-acid media not containing any dextrose. The *B. cereus*, however, made no growth at all with glycocoll, alanine, and phenylalanine and only very little growth with glutamic acid and asparagine, which indicates that this organism is not only incapable of utilizing the carbon in at least the amino acids tested, but can not even make use of their nitrogen. Such a distinctive difference between these two very common groups of soil bacteria, one (the *Bact. fluorescens*) a nonspore-forming organism and the other (*B. cereus*) a spore former, was very unexpected. When these two organisms were inoculated into the synthetic medium containing purified casein, the exactly reverse phenomenon took place —the *B. cereus* developed very rapidly, accompanied by a vigorous decomposition of the casein, while the *Bact. fluorescens* made only a mere trace of growth without bringing about any noticeable hydrolysis of the casein. Thus one organism proves to be strongly proteolytic, capable of rapidly hydrolyzing proteins (this was found to hold true also for purified vegetable proteins, as will be shown later), but unable to attack some simple amino acids; the other is unable to attack native proteins, but is capable of making a very abundant growth on the protein cleavage products—the amino acids.

In view of the fact that these two organisms do not occur in nature in pure culture but in constant association, and the further fact that one can readily utilize the products of the other, the idea suggested itself that proteins could more rapidly be reduced to the ammonia stage by the combined action of these two organisms, one utilizing the products of the other. This has actually been found to be the case, as will be pointed out later.

Another interesting phenomenon was observed in the case of the fungi. Whenever pure amino acids have been used as the only source of carbon and nitrogen, the fungi made a rather limited growth, as shown in Tables I to IV; in most cases not over 50 mg. per 100 c. c. of medium. One could, therefore, be led to a hasty conclusion that fungi can not readily utilize amino acids as a source of carbon. On close examination of the data, however, a more appropriate explanation of this phenomenon is found. In all cases whenever amino acids have been used as sources of carbon and nitrogen, the reaction is changed to alkaline, owing to the rapid accumulation of ammonia. In view of

the fact that the initial reaction of the medium was about pH 7.0 to 7.3 and the medium is not very highly buffered, the reaction changed rapidly to pH 8.0 and even 8.6, which seems to be about the limiting reaction for the growth of at least the two fungi studied. In one case (with 1 per cent glutamic acid) the reaction was left by mistake unadjusted at a pH of 3.0. The bacteria and Actinomyces made no growth, since the reaction was too acid for their development, but the two fungi made a very excellent growth. This shows that there are various factors to be considered in the study of utilization of organic substances under artificial conditions, especially by pure cultures of microorganisms. Notwithstanding the statements made that fungi are not affected by wide ranges in reaction, there is no doubt that, starting with a reaction of about pH 6.0 to 7.0, they will be more favored by the presence of substances which result in products tending to make the reaction acid rather than alkaline; and in the presence of alkaline-forming substances they will be more favored by an acid than by an alkaline or even a neutral reaction.

In connection with the utilization of racemic compounds by microorganisms, attention should be called to the extensive literature on this subject since the work of Pasteur. According to Ehrlich (*6*), *dl*-proline, *dl*-aspartic acid and *dl*-tyrosine are acted upon by yeasts symmetrically, whereas *dl*-alanine, *dl*-glutamic acid and others are acted upon asymmetrically. Pringsheim (*20*) as well as Neuberg (*19*) found that *dl*-glutamic acid and *dl*-aspartic acid are acted upon by fungi and bacteria symmetrically. Various other investigators have shown that, while some organisms attack some amino acids symmetrically, other organisms attack the same acids asymmetrically, depending on the nature of the compound and the organism.

DECOMPOSITION OF GLYCOCOLL (GLYCINE)

The chemical formula for glycocoll is $CH_2.(NH_2).COOH$; in other words, it contains theoretically 18.67 per cent nitrogen and 32.0 per cent of carbon, the ratio between the carbon and nitrogen being 1.7. According to Levene and Van Slyke (*16*), the amount of amino acid obtained for glycocoll, by the Van Slyke method, is somewhat higher than its actual nitrogen content; the figures obtained for amino nitrogen in the case of glycocoll should, therefore, be multiplied by 0.93 to obtain correct values. Somewhat higher results for the NH_2-N in the case of this amino acid were actually obtained in our studies and the results were adjusted by multiplying the figures by 0.93. The growth of only two organisms on glycocoll, in the absence of dextrose, is reported: Trichoderma and Actinomyces (Table I). These two organisms used up, in 12 and 15 days respectively, 29.8 and 41.1 mg. of the amino acid nitrogen and synthesized 50 and 59 mg. of dry mycelium. The Trichoderma produced ammonia equivalent to 24.28 mg. of nitrogen, or nearly 82 per cent of the amino acid decomposed; the Actinomyces produced 30.46 mg. ammonia nitrogen, or 74 per cent of the amino acid decomposed. (The figures for NH_2-N include also the NH_2-N found in the control.) The Trichoderma assimilated over 44 per cent of the carbon decomposed (assuming that the mycelium contains 45 per cent carbon), and the Actinomyces about 38 per cent.

Where dextrose is present the question of carbon and nitrogen utilization by the organisms is somewhat more complicated. The Zygorhynchus decomposed, in 6 days, 22.7 mg. of the glycocoll nitrogen; and, in 10 days, 42.1 mg. In view of the excess of dextrose, most of the nitrogen has been reassimilated by the organism (17.63 mg. out of the 22.7 mg. decomposed) and a small amount is left as ammonia; in 10 days all the dextrose has been used up, and the organism is forced to attack the amino acid molecule for its carbon, which is the reason for the more rapid decomposition and greater accumulation of ammonia. The same is true even to a more striking extent in the case of the Trichoderma. This organism has decomposed, in 5 days, glycocoll equivalent to only 48.6 mg. of nitrogen, 45.66 mg. of which has been reassimilated and resynthesized into fungous mycelium; only a trace of ammonia is found. However, after 10 days, glycocoll equivalent to 108.2 mg. of nitrogen is decomposed, 49.03 mg. is found in the mycelium and 42.80 mg. as ammonia. This is due to the fact that, after the organism has used up all the dextrose, it has to decompose the glycocoll as a source of carbon; so much so that the mycelium actually began to undergo autolysis, as indicated by the diminishing weight. The fact that the nitrogen content of the mycelium did not decrease, while the total weight did, indicates that, after all the dextrose in the medium has been used up the organism will utilize as a source of energy certain carbohydrates

TABLE I.—*The chemistry of decomposition of 1 per cent glycocoll (glycine) by microorganisms, in the presence and absence of dextrose*

Organism used	Dextrose	Age of culture	Total N in 100 c. c.		NH_3—N in 100 c. c.		NH_3—N in 100 c. c.	Dextrose in 100 c. c.		Dry growth		pH
			Found	Decrease from control	Found	Decrease from control		Found	Utilized	Weight	Nitrogen content	
	Per cent	*Days*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Control	None.	------	180.3	-------	178.1	-------	3.54	-------	-------	-------	-------	7.1
Do	2	------	180.3	-------	178.1	-------	3.54	1,870	-------	-------	-------	6.5
Zygorhynchus	2	6	162.8	17.5	155.4	22.7	4.40	380	1,490	251	17.63	6.4
Do	2	10	146.8	33.5	129.0	42.1	13.42	0	1,870	473	27.74	7.2
Trichoderma	None.	6	177.0	3.3	167.4	6.2	7.98	-------	-------	30	3.10	7.8
Do	None.	12	175.0	5.3	148.3	29.8	24.28	-------	-------	50	3.30	8.1
Do	2	5	129.7	50.6	125.5	48.6	1.32	380	1,490	804	45.56	6.7
Do	2	10	122.8	57.5	69.9	108.2	42.80	0	1,870	669	49.03	7.8
Actinomyces	None.	8	178.7	1.6	172.0	4.1	6.36	-------	-------	23	1.4	7.7
Do	None.	15	167.2	13.1	137.0	41.1	30.46	-------	-------	59	4.0	8.0
Do	2	8	157.7	22.6	135.1	36.0	18.84	1,300	570	213	17.5	7.0
Do	2	15	142.0	38.3	108.8	62.3	30.62	1,110	760	303	29.0	7.4
Bact. fluorescens *	None.	9	174.9	5.4	168.0	10.1	6.36	-------	-------	(?)	(?)	7.5
Do	2	5	163.1	17.2	151.8	26.3	2.56	1,050	820	99	10.86	6.6
Do	2	9	(?)	-------	137.2	40.9	7.00	340	1,530	123	18.60	6.4

* Pellicle only incompletely removed.

stored away in its own mycelium in preference to attacking the amino acid; possibly after half of the amino acid has been used up (the natural *d*-form) the remaining *l*-form is attacked with greater difficulty.

In the case of the two fungi, dextrose, as an available carbohydrate, had a sparing action upon the decomposition of the amino acid as well as upon the accumulation of the ammonia. The amino acid is not decomposed as a source of energy and the ammonia does not accumulate (at least not in appreciable amounts) as long as available energy, in the form of dextrose, is present. Actually a larger amount of the amino acid may be decomposed, even in the presence of dextrose; but all the available nitrogen is reassimilated by the organism, so that there is no ammonia left as a waste product.

The Actinomyces, however, decomposed the amino acid and allowed an appreciable accumulation of ammonia, even in the presence of dextrose. After 15 days' incubation, 1,110 mg. of dextrose was still left in the medium, while over 30 mg. of nitrogen as ammonia had already accumulated, owing to the fact that actinomycetes readily utilize amino acids and proteins as sources of carbon or energy; they also prefer these as sources of nitrogen to the ammonia, which accumulates in the medium, even in the presence of dextrose. In this case, then, one can not speak of any sparing action of carbohydrates over proteins, nor can one even justify any theory of "fermentation in preference to putrefaction," meaning, of course, that microorganisms utilize more readily available carbohydrates as sources of energy than they do proteins. It does apply at least to the particular Actinomyces.

The bacterium (*Bact. fluorescens*) behaved similarly to the fungi, decomposing the amino acid in the presence of dextrose only to a limited extent and allowing the accumulation of only small quantities of ammonia, as long as the dextrose lasted in the medium. Unfortunately, it was not possible to obtain the proper weight of the cells for this organism, since it made a rather scanty growth in the glycocoll medium free from dextrose. Blanchetière (*2*) found that this organism transformed 81 per cent of the amino-acid nitrogen into ammonia in 55 days, in the absence of dextrose.

The change in reaction depended entirely on the substances that were being changed. In the absence of dextrose the reaction changed, in all cases, to more alkaline, due to the formation of ammonia; in the presence of dextrose the reaction either did not change or became slightly acid; as soon as the dextrose was used up, or, in the case of the Actinomyces, with the continued decomposition of the glycocoll, even in the presence of dextrose, the reaction became alkaline.

Just how do microorganisms utilize an amino acid as a source of energy? Nawiasky (*18*) suggested the following group of reactions:

TABLE II.—*The chemistry of decomposition of 1 per cent alanine by microorganisms, in the presence and absence of dextrose*

Organism used	Dextrose	Age of culture	Total N in 100 c. c.		NH_3–N in 100 c. c.		NH_3–N in 100 c. c.	Dextrose in 100 c. c.		Dry growth		pH
			Found	Decrease from control	Found	Decrease from control		Found	Utilized	Weight	Nitrogen content	
	Per cent	*Days*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Control	None.		153.5		151.5		2.36					7.1
Do	2		153.5		151.5		2.36	1,760				6.8
Zygorhynchus	None.	12	146.0	7.5	129.47	22.17	6.40			48	3.86	7.8
Do	2	12	141.3	12.2	120.27	31.37	1.12	570	1,190	175	9.29	5.6
Actinomyces	None.	17	125.1	28.4	64.0	87.5	39.17			126	8.97	8.6
Do	2	17	144.4	9.1	105.4	46.1	6.82	1,230	530	82	7.58	4.9
Bact. fluorescens	None.	5			70.0	81.5	32.82					8.8
Do	2	5			92.8	58.7	1.72	750	1,010			4.8
Control	None.		147.6		145.3		3.40					7.3
Do	2		147.6		145.3		3.40	1,680				6.8
Trichoderma	None.	6	135.2	12.4	107.5	37.8	21.98			80	6.97	8.3
Do	2	6	124.4	23.2	107.6	37.7	7.64	400	1,280	562	24.32	5.2

(1) $R.CH(NH_2).COOH+2H=R.CH_2.COOH+NH_3$

$R.CH_2.COOH+3O=R.COOH+CO_2+H_2O$

$R.COOH=R\ H+CO_2$

The first step in the reaction is the splitting off of the ammonia (reductive deaminization), followed by the gradual decomposition of the nitrogen-free molecule, resulting in the liberation of energy and CO_2. The reaction may also take place as follows:

(2) $R.CH(NH_2).COOH=R.CH_2.NH_2+CO_2$

(3) $R.CH(NH_2).COOH+H_2O=R.CHOH.COOH+NH_3$

In reaction (2) we have the formation of an amine, as an intermediary step, and liberation of energy and CO_2. In the third reaction we again have deaminization, by means of hydrolysis, with the formation of an oxy-acid.

Nawiasky found that *B. proteus* is capable of attacking glycocoll to a limited extent, changing a part of it to acetic acid.

$CH_2(NH_2).COOH+2H=CH_3COOH+NH_3$

A part of the acetic acid is probably utilized as a source of energy, in the absence of an available carbohydrate.

DECOMPOSITION OF ALANINE

The chemical formula for alanine is $CH_3.CH.(NH_2).COOH$; it contains 15.73 per cent nitrogen and 40.4 per cent carbon, or 2.57 times as much carbon as nitrogen. The Trichoderma decomposed alanine equivalent to 37.8 mg. of nitrogen, in the absence of dextrose (Table II); out of this, 21.98 mg. has been changed to ammonia and 6.97 mg. has been reassimilated. The organism synthesized 80 mg. of mycelium, which contains about 36 mg. of carbon; the amino acid decomposed contained $37.8\times2.57=97.15$ mg. of carbon; in other words, the organism reassimilated about 37 per cent of the carbon that it has decomposed. About the same amount of alanine has been decomposed by the Trichoderma also in the presence of dextrose, but a much smaller amount of the nitrogen is found as ammonia and a much larger quantity of it was reassimilated. The process of carbon utilization in the presence of dextrose is

$$\frac{562\times45\text{ per cent}}{1{,}280\times40\text{ per cent}+37.7\times2.57}=41.5$$

per cent. The organism is somewhat more efficient in synthesizing its protoplasm, in the presence of an available carbohydrate, requires less energy, and assimilates less nitrogen per unit of protoplasm synthesized.

The Actinomyces synthesized 126 mg. mycelium from an amount of alanine decomposed which is equivalent to 87.5 mg. of nitrogen; the efficiency of this organism is $\frac{126\times45\text{ per cent}}{87.5\times2.57}=25.1$ per cent. In the presence of dextrose, the Actinomyces decomposed alanine, equivalent to 46.1 mg. of nitrogen, and 530 mg. of dextrose, but synthesized only 82 mg. of mycelium, showing a lower efficiency. This tends again to bring out that the Actinomyces may prefer

amino acids and protiens as sources of energy, even in the presence of such an available carbohydrate as dextrose.

The development of *Bact. fluorescens* in the alanine medium was very similar to its growth upon glycocoll; that is, a larger amount of amino acid has been decomposed and a much larger amount of ammonia has accumulated in the absence than in the presence of dextrose. The changes in reaction were similar to those observed with glycocoll.

In comparing the amounts of ammonia found in the medium with the amount of growth produced by the organisms upon glycocoll and alanine, as substrates, attention should be called to the following fact: The Trichoderma liberated, in the case of glycocoll, 24.28 mg. nitrogen as ammonia for 50 mg. of mycelium synthesized and, in the case of alanine, 21.98 mg. NH_3-N for 80 mg. of mycelium; the Actinomyces liberated, in the case of glycocoll, 30.46 mg. of NH_3-N for 59 mg. of mycelium and, in the case of alanine, 39.17 mg. NH_3-N for 126 mg. of mycelium. This difference is due not to the more rapid decomposition of the glycocoll (just the reverse is true), but to the fact that the C:N ratio is smaller, in the case of glycocoll than in the case of alanine; the organisms will find more available energy in the latter and will, therefore, assimilate more of the nitrogen and leave a smaller amount of waste as ammonia.

DECOMPOSITION OF PHENYLALANINE

The chemical formula for phenylalanine is $C_6H_5.CH_2.CH\ (NH_2).COOH$; this amino acid contains 8.49 per cent nitrogen, 65.5 per cent total carbon and about 22 per cent carbon, outside of the benzol ring. The Zygorhynchus and Actinomyces decomposed only a small amount of the phenylalanine (Table III); the color of the medium soon became reddish and the odor distinctly aromatic. It is therefore possible that the organism is unable to utilize the various products formed from the decomposition of the phenylalanine, containing the benzol group; their rapid accumulation, especially in the absence of dextrose, may soon stop the further development of the organism. The Trichoderma, however, used up, in 6 days, 37.6 per cent of the phenylalanine in the absence of dextrose and 56.4 per cent in the presence of dextrose. If the efficiency of the organism to assimilate carbon is as great with phenylalanine as with the other amino acids—40 per cent—we could conclude that the Trichoderma attacked also the benzol group; 131 mg. of dry mycelium contains about 60 mg. C, which would indicate about 150 mg. of original carbon decomposed; 37.6 per cent of the phenylalanine which was decomposed in the medium (or 338 mg. of phenylalanine) contained about 220 mg. of total carbon and only about 75 mg. of the carbon outside of the ring.

The same is true of the cultures containing the dextrose. All the nitrogen of the amino acid decomposed was recovered as ammonia and in the mycelium.

Of the two bacteria, *B. cereus* made no growth at all, while *Bact. fluorescens* decomposed the phenylalanine very rapidly, over 55 per cent in 7 days, in the absence of dextrose, thus showing

TABLE III.—*The chemistry of decomposition of 0.9 per cent phenylalanine by microorganisms, in the presence and absence of dextrose*

Organisms used	Dextrose	Age of culture	Total N in 100 c. c.		NH_3-N in 100 c. c.		NH_3-N in 100 c. c.	Dextrose in 100 c. c.		Dry growth		pH
			Found	Decrease from control	Found	Decrease from control		Found	Utilized	Weight	Nitrogen content	
	Per cent	*Days*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Control	None		79.80		73.96		Tr.					7.1
Do.	2		79.80		73.96		Tr.	1,700				6.5
Zygorhynchus	None	7	77.90	1.90	66.36	7.60	8.56			35	2.4	7.7
Do.	2	7	66.10	13.70	61.08	12.88	2.98	370	1,330	272	14.00	5.2
Trichoderma	None	6	70.00	9.80	46.16	27.80	17.94			131	9.78	7.8
Do.	2	6	39.20	40.60	32.20	41.76	4.22	325	1,375	654	37.12	6.0
Actinomyces	None	26								30	2.5	7.8
Do.	2	26						1,640	60	42	2.2	6.2
Bact. fluorescens.	None	7			32.56	41.40	25.40					8.1
Do.	2	7			52.60	21.36	4.86	760	940			4.4

that this organism is capable of utilizing both the carbon and nitrogen of the particular amino acid.

Nawiasky (*18*) also found that *B. proteus* was capable of decomposing phenylalanine, with the liberation of 28.09 per cent of the nitrogen as ammonia, in 6 days. Among the products resulting from the decomposition of the phenylalanine, benzoic acid, phenylacetic acid, phenylpropionic acid, and phenyl-ethylamine were demonstrated, all benzol-ring compounds.

Blanchetière (*2*), however, found that *Bact. fluorescens liquefaciens* transformed only 6.4 per cent of the nitrogen of phenylalanine into ammonia, in 7 days, and 60.7 per cent in 43 days. Since no phenol or benzoic acid could be demonstrated in the medium, Blanchetière concluded that the organism is capable of breaking down the benzol ring.

DECOMPOSITION OF GLUTAMIC ACID

The chemical formula for glutamic acid is $HOOC.CH_2.CH_2.CH(NH_2)COOH$. This amino acid therefore contains 9.52 per cent nitrogen and 40.8 per cent of carbon, 4.28 being the ratio between the carbon and the nitrogen.

The content of amino acid actually found in the solution (Table IV) was lower than the theoretical amount. This is probably owing to the small amount of HCl present as an impurity. The Zygorhynchus decomposed 67 per cent of the amino acid, in the absence of dextrose, in 10 days, and the Trichoderma 63 per cent in 7 days; about half of the nitrogen was liberated as ammonia, about one quarter of the nitrogen was reassimilated by the organisms, and about a quarter of the nitrogen was present in solution in some different form. It has either been changed into another nitrogen compound, nonamino in nature, or it is a result of autolysis, which leads to a decrease in the nitrogen content of the mycelium and an increase of the organic and ammonia nitrogen in solution; the organic nitrogen secreted consists of various amino acids and hexone and purin bases, as shown by Reed (*21*) for Glomerella. It is probable that the discrepancy between the total nitrogen in solution and the sum of amino and ammonia nitrogen is due largely to the formation of other nitrogenous substances. This is brought out by the fact that discrepancies between the total nitrogen in solution and that accounted for by the residual amino acid and ammonia were often observed in the case of other amino acids. None seemed to show so large a difference as that found in the case of the glutamic acid.

The synthesis of mycelium was also much more extensive with this amino

TABLE IV.—*The chemistry of decomposition of 1 per cent glutamic acid by microorganisms, in the presence and absence of dextrose*

Organism used	Dextrose	Age of culture	Total N in 100 c. c.		NH_3—in 100 c. c.		NH_3—N in 100 c. c.	Dextrose in 100 c. c.		Dry growth		pH
			Found	Decrease from control	Found	Decrease from control		Found	Utilized	Weight	Nitrogen content	
	Per cent	*Days*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Control	None.		89.12		89.02		Tr.					3.0 [a](6.3)
Do	2		89.12		86.32		Tr.	1,815				3.0 [a](6.3)
Zygorhynchus	None.	10	75.96	13.16	29.44	59.58	25.4			204	13.65	5.7
Do	2	10	46.84	42.28	12.08	74.24	18.3	0	1,815	853	40.18	4.5
Trichoderma	None.	7	67.97	21.15	32.88	56.14	29.12			218	14.28	7.6
Do	2	7	58.75	30.37	47.26	39.06	5.48	460	1,355	545	29.12	3.4
Actinomyces	None.	13	70.80	18.32	6.28	82.74	28.36			169	13.44	9.5
Do	2	13	50.02	39.10	3.54	82.78	12.34	525	1,290	488	39.20	9.0
B. cereus	None.	15			64.68	24.34	5.16					8.8
Do	2	15			68.04	18.28	2.38	1,640	175			4.9
Bact. fluorescens	None.	5			15.43	73.59	28.50			128	12.02	9.1
Do	2	5			14.88	71.44	6.10	705	1,110	328	32.88	8.3

[a] Control for *Actinomyces*, *B. cereus*, and *Bact. fluorescens*.

acid than with the other three acids previously studied. This is attributable, on the one hand, to the much larger ratio of the carbon to the nitrogen in the particular amino acid, and, on the other, to the fact that glutamic acid is very favorable to respiration, resulting in the formation of very little volatile acid. Nawiasky (*18*) found that *B. proteus* will transform, in 3 days, 11.67 per cent of the nitrogen of glutamic acid to ammonia and, in 15 days, 52.97 per cent. The amount of ammonia, in comparison with the amount of mycelium synthesized is smaller in the case of the glutamic acid than in the case of glycocoll or alanine, as shown in the following summary:

Amino acid	C/N	Organism	Growth	NH_3–N	Growth / NH_3–N
			Mg.	*Mg.*	
Glycocoll	1.7	Trichoderma	50	24.28	2.0
Do	1.7	Actinomyces	59	30.46	2.0
Alanine	2.57	Zygorhynchus	48	6.40	7.5
Do	2.57	Trichoderma	80	21.98	3.6
Do	2.57	Actinomyces	126	39.17	3.2
Glutamic acid	4.28	Zygorhynchus	204	25.40	8.0
Do	4.28	Trichoderma	218	29.12	7.5
Do	4.28	Actinomyces	169	28.36	5.9
Do	4.28	B. fluorescens	128	28.50	4.5

The higher the ratio of carbon to nitrogen in the amino acid molecule, the less is the amount of ammonia produced per unit of protoplasm synthesized. This is based upon the simple principle that ammonia is a waste product in the carbon metabolism of an organism, with proteins or their derivatives as sources of energy; the amount of ammonia accumulated in the medium depends upon the growth of the organism and particularly upon the ratio of the available carbon to the available nitrogen in the medium; this holds true also when an available carbohydrate, like dextrose, is present in the medium; when the amount of available carbon is increased, the amount of ammonia accumulation, under the same conditions, will be decreased.

It is interesting to point in this connection to the work of Jodidi (*10*), who found that glycocoll, containing 18.7 per cent nitrogen, was decomposed in the soil and about 80 per cent of the nitrogen was transformed into ammonia, whereas leucine, with 10.7 per cent nitrogen, gave only 50 per cent of its nitrogen as ammonia. This is exactly what one might expect from the above considerations, since the C/N of glycocoll is 1.7 and the C/N of leucine is 5.1. The explanation given by Jodidi was that "the slower formation of ammonia from leucine is to be ascribed to the inert paraffin character of the comparatively long hydrocarbon chain, etc." Jodidi was correct, however, in respect to phenylalanine, which produced even less ammonia than the leucine, owing to the resistant character of its benzene ring.

Kelley (*11*) also brought out the fact that the ammonia content of the medium is definitely affected by its carbon content. The same amount of ammonia was formed from casein, soybean cake, cottonseed meal and linseed meal, when enough starch was added to each, so as to have the same amount of nitrogenous and nonnitrogenous substances, the carbon-nitrogen ratio actually affecting the ammonia formation in soils.

By comparing the ammonia formed by the three groups of organisms—the fungi, actinomycetes, and bacteria—an increasing amount of ammonia is found to be formed per unit of protoplasm synthesized, in order named. This holds true in spite of the fact that fungi contain the smallest percentage of nitrogen, and can be explained only by a proper understanding of the metabolism of these three groups of organisms. The fungi reassimilate the largest proportion of carbon; therefore they will also reassimilate a proportionately larger amount of nitrogen and leave a proportionately smaller amount of waste nitrogen as ammonia.

The actinomycetes, and especially the bacteria, which produce a smaller amount of growth, use the available energy less economically; they assimilate less of the nitrogen, waste more of the carbon (as CO_2), and therefore liberate a larger amount of ammonia, as shown in the summary taken from Table IV (shown on page 273).

For approximately the same amount of ammonia formed, the fungi decomposed less amino acid and produced a heavier growth than the Actinomyces and Bacterium.

SUMMARY

Organism	Amino acid decomposed	Growth	NH_3–N
	Mg.	*Mg.*	*Mg.*
Zygorhynchus	59.58	204	13.65
Trichoderma	56.14	218	14.28
Actinomyces	82.74	169	13.44
Bact. fluorescens	73.59	128	12.02

In the presence of dextrose in the medium, the same process took place as with the other amino acids: A larger amount of protoplasm was synthesized; a larger amount of nitrogen was reassimilated, and a smaller amount of ammonia was left in the medium. The Actinomyces is again an exception; it continued to decompose the amino acid with the liberation of ammonia,

TABLE V.—*The chemistry of decomposition of 1.4 per cent glutamic acid by microorganisms, in the presence and absence of dextrose*

Organism used	Dextrose	Age of culture	Total N in 100 c. c.		NH_3–N in 100 c. c.		NH_3–N in 100 c. c.	Dextrose in 100 c. c.		Dry growth		pH
			Found	Decrease from control	Found	Decrease from control		Found	Utilized	Weight	Nitrogen content	
	Per cent	*Days*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Control	None.	------	126.40	------	126.40	------	------	------	------	------	------	7.3
Do	2	------	126.40	------	126.40	------	------	1,800	------	------	------	6.4
Zygorhynchus	None.	15	------	------	122.24	4.16	3.62	------	------	30	2.6	8.4
Do	2	15	------	------	104.96	21.44	4.12	65	1,735	473	17.92	7.1
Trichoderma	None.	13	------	------	122.60	3.80	1.76	------	------	28	2.0	8.2
Do	2	13	------	------	94.64	31.76	3.00	155	1,645	627	30.49	6.9
Actinomyces	None.	19	108.08	18.32	61.46	64.96	34.04	------	------	141	11.60	9.8
Do	2	19	100.16	26.24	89.49	36.91	9.18	1,080	720	350	27.20	8.7
B. cereus	None.	7	------	------	113.78	12.62	0.52	------	------	------	------	7.6
Do	2	7	------	------	117.04	9.36	1.76	1,710	90	------	------	5.8
Bact. fluorescens	None.	7	86.03	40.37	28.91	97.49	22.89	------	------	312	32.32	9.4
Do	2	7	48.35	78.05	13.68	112.62	9.36	525	1,275	831	78.48	8.9

TABLE VI.—*The chemistry of decomposition of 1 per cent asparagine by microorganisms, in the presence and absence of dextrose*

Organism used	Dextrose	Age of culture	Total N in 100 c. c.		NH_3–N in 100 c. c.		NH_3–N in 100 c. c.	Dextrose in 100 c. c.		Dry growth		pH
			Found	Decrease from control	Found	Decrease from control		Found	Utilized	Weight	Nitrogen content	
	Per cent	*Days.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Control	None.	------	179.5	------	90.9	------	Trace.	------	------	------	------	7.0
Do	2	------	179.5	------	93.0	------	Trace.	1,840	------	------	------	6.4
Zygorhynchus	None.	6	177.0	2.5	------	------	50.40	------	------	21	1.6	8.0
Do	None.	9	174.0	5.5	73.4	17.5	69.56	------	------	39	3.04	8.6
Do	2	6	142.0	37.5	71.5	21.5	31.30	60	1,780	321	25.5	7.0
Do	2	9	155.0	24.5	56.3	36.7	83.56	0	1,840	223	13.44	7.7
Trichoderma	None.	4	174.0	5.5	80.8	10.1	8.31	------	------	18	------	7.5
Do	None.	12	174.0	5.5	84.7	6.2	74.66	------	------	16	------	8.6
Do	2	4	137.0	42.5	67.2	25.8	3.81	670	1,170	643	39.86	6.4
Do	2	12	134.0	45.5	48.3	44.7	65.12	0	1,840	502	36.20	7.9
Actinomyces	None.	22	169.1	10.4	77.2	13.7	18.48	------	------	41	2.83	7.7
Do	2	9	159.5	20.0	78.9	14.1	5.76	1,070	770	176.5	17.87	6.3
Do	2	22	148.4	31.1	19.6	73.4	65.76	0	1,840	249	22.58	8.4
Do	2	22	164.4	15.1	71.6	21.4	4.48	940	900	167	14.57	6.0
B. cereus	None.	13	150.0	29.5	56.2	34.7	55.54	------	------	17	------	8.6
Do	2	13	174.0	5.5	72.0	20.8	30.64	1,560	280	------	------	6.2
Bact. fluorescens	None.	3	156.0	23.5	69.9	21.0	86.80	------	------	53	6.17	8.6
Do	None.	7	113.0	66.5	28.5	62.4	91.90	------	------	------	------	8.6
Do	2	3	151.0	28.5	66.2	26.8	78.50	1,340	500	322	28.64	8.0
Do	2	7	108.0	71.5	37.0	56.0	64.50	50	1,790	Lost.	------	7.8

showing that this organism will also prefer glutamic acid as a source of energy, even in the presence of dextrose.

Table V contains only some confirmatory data. Owing to the alkaline reaction, the fungi were soon injured in their development, in the absence of dextrose, by the rapidly accumulating ammonia. The Actinomyces and the bacteria, however, that can stand a higher alkalinity than the fungi, decomposed a great deal of the glutamic acid, even in the 1.4 per cent concentration, with results similar to those obtained in the previous experiment.

DECOMPOSITION OF ASPARAGINE

The chemical formula for asparagine is $NH_2.CO.CH_2.CH(NH_2)COOH(+H_2O)$; it contains 18.68 per cent nitrogen and 32 per cent carbon, the ratio of carbon to nitrogen being 1.7, very similar to glycocoll. However, the two NH_2 groups in the asparagine molecule are in different positions, one as an amino and the other as an amide group. The various references in the literature on the decomposition of asparagine tend to indicate that the splitting off of these two groups and their transformation into ammonia take place with unequal rapidity.

The asparagine is first changed to aspartic acid and ammonia, then to succinic acid; the aspartic acid may also change to acetic acid:

$$NH_2.CO.CH_2.CH(NH_2)COOH+H_2O=HOOC.CH_2.CH(NH_2)COOH+NH_3$$

$$HOOC.CH_2.CH(NH_2)COOH\ (CH_2COOH)_2+NH_3$$

$$(CH_2COOH)_2+7O=4\ CO_2+3H_2O$$

Nawiasky (*18*) found that *B. proteus* reduces, in 24 hours, almost all the asparagine to aspartic acid and ammonia. The aspartic acid changes, at the same time, to succinic or acetic acids and ammonia.

In our own experiments (Table VI), Zygorhynchus decomposed, in 9 days, in the absence of dextrose, 17.5 mg. amino nitrogen out of a total of 90.9 mg.; however, there were 69.56 mg. of ammonia nitrogen in the medium, showing that the largest amount of ammonia originates from the amide group. The amount of growth produced by both fungi is very small, in comparison with the amount of ammonia formed and is more comparable with the decrease in the amino acid content. This points to two things: (1) The amide group is split off more readily than the amino group (the latter only as far as the aspartic acid is decomposed as a source of energy); (2) the rapid change in reaction to alkalinity, due to the accumulation of ammonia, represses the further development of the fungi and the amount of growth as well as amino acid decomposed is limited. The amount of available carbon in the asparagine molecule is so small, in comparison with the available nitrogen, that only a small amount of growth may be formed. The Actinomyces produced 41 mg. of growth, with a comparatively small amount of amino nitrogen decomposed and ammonia formed, in the absence of dextrose. The largest amount of ammonia was produced by the *Bact. fluorescens*: 86.80 mg. of ammonia nitrogen was found in the medium after 3 days, while only 21.0 mg. of amino nitrogen had been decomposed. The fact that large amounts of ammonia are formed in the medium even in the presence of dextrose indicates that, although ammonia formation from the amino acid is a direct result of metabolism, the formation of ammonia from an amide may be primarily a result of enzyme action, regardless of whether the ammonia is needed by the organism. This is further substantiated by the fact that the amount of ammonia rapidly increases with age of culture, independent of the actual carbon assimilation.

The addition of dextrose tends to keep down the amount of ammonia accumulated in the medium, especially in the case of the fungi as a result of the probable reassimilation of the ammonia as long as there is available energy. However, as soon as the energy is used up, the ammonia begins to accumulate rapidly. This is clearly seen in the case of the Actinomyces, for example. After 9 days' incubation, only 770 mg. of dextrose was decomposed, 176.5 mg. of mycelium was resynthesized, and only 5.76 mg. ammonia were found in the medium. After 22 days, the duplicate cultures showed different results; one made further growth and the other did not develop any further than at the first period of analysis. It is interesting to note that the culture containing some undecomposed dextrose, after 22 days, showed about the same relationship in growth, reaction, decomposition of amino nitrogen and ammonia formation as the 9-day-old culture. In the second flask, however, where all the dextrose had disappeared, the decomposition of the amino acid advanced rapidly, accompanied by ammonia accumulation and change in reaction. The Actinomyces seemed to

have produced all or most of its ammonia from the amino acid group and not from the amide group, while the *Bact. fluorescens* seemed to have produced most of its ammonia from the amide and not from the amino acid group. This again goes to emphasize that the decomposition of proteins and amino acids and accumulation of ammonia are results of the metabolism of the particular microorganisms, under specific conditions. The changes produced as a result of decomposition of the protein and amino acid molecules do not depend so much upon the age of the culture as upon its development; that is, a more abundant growth in a shorter period of time will produce a series of changes parallel to its growth rather than its age.

The *B. cereus* made only a very limited growth with asparagine; in the rapidity of deamidization it behaved similarly to the *Bact. fluorescens*.

CHEMISTRY OF DECOMPOSITION OF CASEIN

Casein is soluble in dilute alkali solutions and can therefore be readily used as a source of nitrogen and carbon for the activities of microorganisms. Only a small amount of the casein nitrogen is present in the lysin group, which accounts for the small amount of amino nitrogen present in the control solution. The two fungi and Actinomyces readily attacked the casein; the two bacteria behaved, however, in an exactly reverse manner than they did in respect to the amino acids. The *B. cereus* was very active and *Bact. fluorescens* hardly attacked the casein at all.

The Zygorhynchus is capable of decomposing casein, but very little amino nitrogen accumulates in the medium, showing that it decomposes the amino acids as soon as they are formed. One would almost expect this to occur, since this organism was capable of utilizing, as a source of carbon and nitrogen, all the amino acids previously tested, if only present in the proper concentration and with the proper reaction. The casein as such was all decomposed, since no precipitate was obtained any longer with acetic acid. It was not, however, completely decomposed, even in the presence of dextrose, to carbon dioxide, water, and ammonia, as seen from the fact that, although 120.5 mg. of the nitrogen was present in solution, only 17 mg. of it was found in the form of amino and ammonia nitrogen. In other words, a large part of the nitrogen was left in the form of polypeptides and other protein derivatives, which the organism could not very readily and rapidly utilize as a source of carbon. In the presence of available dextrose, a large part of the

TABLE VII.—*The chemistry of decomposition of 1 per cent casein by microorganisms, in the presence and absence of dextrose*

Organism used	Dextrose	Age of culture	Total N in 100 c. c.		NH_3—N in 100 c. c.		NH_3—N in 100 c. c.	Dextrose in 100 c. c.		Dry growth		pH
			Found	Decrease from control	Found	Increase over control		Found	Utilized	Weight	Nitrogen content	
	Per cent	*Days*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	
Control	None.	------	126.4	-------	6.84	-------	2.10	-------	-------	-------	-------	7.0
Do	2	------	126.4	-------	6.84	-------	2.10	1,890	-------	-------	-------	6.4
Zygorhynchus	None.	16	120.5	5.9	10.32	3.48	13.52	-------	-------	93	6.35	7.8
Do	2	16	76.1	50.3	9.48	2.64	19.82	0	1,890	643	40.95	7.8
Trichoderma	None.	6	113.1	13.3	26.24	19.40	14.14	-------	-------	154	14.99	7.5
Do	2	6	70.7?	55.7?	15.76	8.92	3.00	560	1,330	452	19.90	4.6
Do [a]	None.	5	-------	-------	26.17	-------	3.31	-------	-------	59	-------	7.2
Do [a]	None.	11	-------	-------	40.02	-------	24.29	-------	-------	200	19.62	8.2
Do [a]	2	5	-------	-------	16.94	-------	1.66	[b]1,385	335	92	-------	5.5
Do [a]	2	11	-------	-------	21.90	-------	9.11	[b]40	1,680	692	44.90	6.6
Actinomyces	None.	19	108.9	17.5	8.94	2.10	31.80	-------	-------	117	9.95	8.0
Do	2	19	104.2	22.2	15.40	8.36	3.00	1,380	510	-------	-------	5.1
B. cereus	None.	10	101.1	25.3	18.10	11.26	45.50	-------	-------	102	8.07	8.4
Do	2	10	120.0	6.4	9.75	2.91	3.30	1,700	190	-------	-------	4.8
Do [a]	None.	6	-------	-------	54.04	-------	24.92	-------	-------	66	-------	8.5
Do [a]	None.	13	-------	-------	24.77	-------	38.52	-------	-------	156	-------	8.6
Do [a]	2	6	-------	-------	25.91	-------	2.8	[b]1,490	230	-------	-------	4.7
Bact. fluorescens.	None.	13	124.3	2.1	8.34	1.50	2.36	-------	-------	-------	-------	7.1
Do	2	13	105.8	20.6	7.78	0.94	1.12	1,580	310	-------	-------	4.6

[a] Data from a different experiment.

[b] Control in this case 1,720 mgm.

nitrogen was reassimilated by the organism and, after all the dextrose was used up, ammonia began to accumulate in the medium; only a small part of the nitrogen was present in this case in the form of polypeptides.

Similar results were obtained with the Trichoderma. In the absence of dextrose, the organism produced a more abundant growth than the Zygorhynchus, and it also changed about 40 per cent of the nitrogen in solution to amino nitrogen and ammonia. The fact that Trichoderma is capable of breaking down proteins more vigorously than Zygorhynchus is further indicated by repeated experiments on casein decomposition: After 11 days, there was present in the medium 40.02 mg. of amino nitrogen and 24.29 mg. of ammonia nitrogen, or nearly 60 per cent of the total nitrogen. Dextrose exerts a protective action, both on the hydrolysis of the protein and upon ammonia formation, similar to the phenomena elucidated above. As long as there is dextrose left in the medium, the reaction will be acid (causing a precipitation of the casein, followed by its dissolution as soon as the dextrose is used up), and very little ammonia will accumulate.

The Actinomyces behaved in as imilar way. There was, however, an increase in the amino nitrogen content in the dextrose containing media; the ammonia accumulated largely in the dextrose-free media.

B. cereus attacked casein more vigorously in the absence of dextrose than in its presence. There was at first a rapid increase in the amino nitrogen content in the absence of dextrose followed by a decrease, probably owing to the fact that, after some time, the increase in the amino nitrogen content resulting from the hydrolysis of the protein, then of the protein derivatives, was balanced and finally exceeded by the increase in the ammonia resulting from the decomposition of the amino compounds. It is interesting to note that there was a decided increase in the amino nitrogen content even in the presence of dextrose, confirming the observations of DeBord (*5*), although in this case it was lower than in the absence of dextrose. The ammonia accumulation was insignificant in the presence of dextrose. This would indicate that, although the protein molecule may be hydrolyzed by *B. cereus* even in the presence of dextrose, the hydrolysis does not go much further than the amino acid stage, so that no ammonia can accumulate. In view of the fact that *B. cereus* does not attack readily simple amino acids, at least not those that were tested, one would expect that the hydrolysis of casein by *B. cereus* would lead to an accumulation of certain amino acids; since ammonia results chiefly when the amino acids are used as sources of energy (comparatively little energy being liberated in the hydrolysis of the protein to the amino acids), no ammonia would accumulate in the presence of an available carbohydrate. In the absence of dextrose, the organism has to utilize the amino acids as sources of energy, and the results seem to indicate that *B. cereus* is capable of decomposing at least certain fractions of the protein molecule, when ammonia is formed and allowed to accumulate in the medium.

Bact. fluorescens was found unable to utilize casein, either as a source of carbon or of nitrogen; the fact that small amounts of dextrose were used would lead one to think there were certain substances present in the casein which were utilized by the organisms as sources of nitrogen.

The two bacteria behaved thus in a distinctly different manner toward a protein and its derivatives: *B. cereus* is typically proteolytic, capable of decomposing native proteins (the same was found to hold true in the case of purified vegetable proteins, as will be shown elsewhere), while *Bact. fluorescens* is nonproteolytic but can utilize amino acids (and probably also polypeptides) as sources of energy and nitrogen. By combining the two organisms, one would logically expect that the course of transformation of the protein molecule would take place in a distinctly different manner than by either organism alone. This was actually found to be the case, as shown in Table VIII and Figure 1.

B. cereus alone hydrolyzed the casein with a gradual accumulation of amino nitrogen and ammonia, so that, in 6 days, 35 per cent of the nitrogen in solution was in the form of amino nitrogen and 12 per cent in the form of ammonia. After that the total content of amino nitrogen decreased, owing to its transformation to ammonia, so that, in 15 days, the total amino nitrogen dropped from 44.89 mg. (6 days) to 24.62 mg. (about 30 per cent of the nitrogen in solution); the ammonia rose in that period of time from 16.62 mg. to 46.52 mg., or from 12.5 per cent to over 50 per cent of the nitrogen in solution. This indicates definitely that the hydrolysis of the polypeptides continues even after the maximum amino nitrogen has been reached; only more of it is changed to ammonia.

When *Bact. fluorescens* and *B. cereus* were inoculated into the same solution, the latter decomposed the casein, while the former decomposed a large part of the simple amino compounds, as soon as formed, with the formation of ammonia. The number of cells of *Bact. fluorescens* greatly exceeded those of *B. cereus;* the culture looked and smelled like a typical *Bact. fluorescens* culture. The accumulation of amino nitrogen in the mixed culture never reached as high a point as in the culture of *B. cereus* alone; so that, in 6 days, the mixed culture produced a little more than half as much amino nitrogen, as *B. cereus* alone did, and over twice as much ammonia.

The question often arises in the study of soil microbiology: Just what part is played in the soil by a large number of bacteria and other organisms incapable of decomposing native proteins, celluloses, and other complex compounds added to the soil? It may be that here will be found the answer to this question; namely, that they attack the products resulting from the decomposition of complex compounds by those organisms that are able to do so. Various instances of such associative action are on record as between the nitrate-forming and nitrite-forming bacteria, nitrogen-fixing and cellulose-decomposing bacteria, etc. There is no reason to doubt that a similar associative action is found here between the large spore-forming and other strongly proteolytic bacteria, (aerobic and anaerobic) and fungi, on the one hand, and the small, nonspore forming and nonproteolytic bacteria on the other. This will also explain the apparent discrepancy between the numbers of the spore-forming and nonspore-forming bacteria in the soil, the former being present there only as 5 to 10 per cent of the flora developing on the plate, and the latter as 50 to 80 per cent. This discrepancy led Conn (*4*) to doubt the activity of the spore formers in the soil. The explanation here may again be very simple: The strong proteolytic cells will obtain a comparatively small amount of energy from the hydrolysis of the proteins to amino acids, even when they transform a part of these to ammonia; this and the large size of the cell may account for the fact that only a limited number of cells are capable of

TABLE VIII.—*Decomposition of casein by B. cereus, by Bact. fluorescens and by both organisms in association*

Age of culture	Organism used								
	B. cereus					Bact. fluorescens			
	Total N in 100 c. c. solution	NH_3–N in 100 c. c.	NH_3–N in 100 c. c.	Weight of pellicle	pH	Total N in 100 c. c. solution	NH_3–N in 100 c. c.	NH_3–N in 100 c. c.	pH
	Mg.	*Mg.*	*Mg.*	*Mg.*		*Mg.*	*Mg.*	*Mg.*	
Control	140.6	7.57	2.30	----------	7.1	140.6	7.57	----------	7.1
1 day	----------	9.55	3.30	----------	7.1	----------	6.34	3.00	7.1
2 days	----------	15.30	3.00	----------	7.1	----------	9.86	1.04	7.1
4 days	135.03	29.93	12.94	50	7.4	----------	7.90	1.40	7.1
6 days	128.1	44.89	16.62	73	8.0	----------	8.21	2.06	7.3
9 days	113.2	38.93	40.86	113.2	8.5	----------	7.46	3.00	7.3
15 days	82.56	24.62	46.52	209	8.9	139.6	9.86	0.88	7.3

Age of culture	Organism used—B. cereus+Bact. fluorescens				
	Total N in 100 c. c. solution	NH_3–N in 100 c. c.	NH_3–N in 100 c. c.	Weight of pellicle *	pH
	Mg.	*Mg.*	*Mg.*	*Mg.*	
Control	140.6	7.57	2.30	----------	7.1
1 day	----------	7.26	1.76	----------	7.1
2 days	----------	18.42	7.96	----------	7.2
4 days	119.80	23.31	27.86	156	8.2
6 days	115.50	27.68	35.36	185	8.9
9 days	92.14	13.90	56.76	203	9.4
15 days	91.22	10.84	59.72	224	9.4

* Approximate.

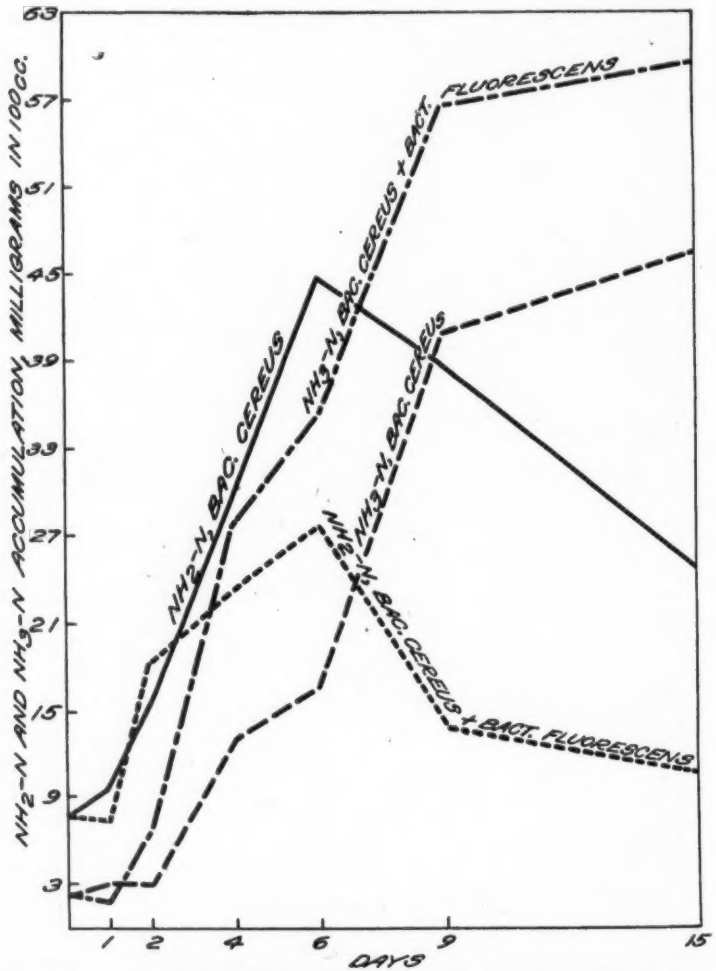

FIG. 1.—Course of formation and accumulation of NH_3—N and NH_2—N in casein solution by *Bac. cereus* and *Bac. cereus*+*Bact. fluorescens*

developing with the amount of energy made available. The small nonspore-forming bacteria, utilizing protein derivatives and breaking these down to ammonia, will obtain a comparatively larger amount of energy; this fact, as well as the smaller size of the cell, may be the cause for the development of a large number of individuals.

It remains to be seen whether this holds true also for other bacteria, or the two bacteria used in this study just happen to be exceptions.

One more point remains to be discussed in this preliminary study on the decomposition of proteins and their derivatives by microorganisms; namely, the influence of carbohydrates upon the decomposition of the proteins and upon ammonia accumulation. It was pointed out above that the theory that "available carbohydrates exert a protective action upon the decomposition of proteins by microorganisms" should be modified, since, as seen in the case of the Actinomyces, some organisms are capable of utilizing proteins as sources of energy, even in the presence of dextrose, probably the most available of all carbohydrates for microorganisms, including actinomycetes. Table IX contains a few data on the influence of other carbohydrates upon the decomposition of casein by bacteria and fungi. Since *B. cereus* is unable to decompose celluloses, a strong cellulose-decomposing bacterium *Bact. fimi* of Kellerman and associates, was used. In the case of *B. cereus*, both cane sugar and starch exerted a protective action upon ammonia accumulation, starch to a lesser extent, possibly because it is not so readily available as the sugars. In the case of Trichoderma, cane sugar and starch also exerted a protective action, while cellulose did not. Trichoderma, one of the strongest cellulose-decomposing organisms ever tested in this laboratory, behaves towards the protein as if the cellulose were not present in the medium at all; in other words, it prefers the protein as a source of energy to the available carbohydrate (cellulose). The *Bact. fimi*, however, decomposed the protein only to a very limited extent and preferred the carbohydrate as a source of energy.

The above theory should therefore be modified to a simple problem of metabolism, which holds true not only for microorganisms but for all living beings that depend for their energy upon complex substances; namely, that a living being derives its energy from a substance, which is most available to it and which may be specific for a particular organism; a large number of microorganisms prefer certain simple available carbohydrates to proteins as sources of energy, and in the presence of those, the proteins will be utilized only as sources of nitrogen; therefore, ammonia, which is chiefly a product of utilization of proteins as sources of energy, will not accumulate.

SUMMARY

A study has been made of the nature of decomposition by two fungi, two bacteria, and one Actinomyces of certain pure amino acids and casein; the latter was used in a synthetic medium as sources of nitrogen alone and of

TABLE IX.—*Influence of various carbohydrates (2 per cent) upon the decomposition of casein by microorganisms* [a]

Carbohydrate	Organism									
	B. cereus			Trichoderma				Bact. fimi		
	NH_3–N in 100 c. c.	NH_3–N in 100 c. c.	pH	NH_3–N in 100 c. c.	NH_3–N in 100 c. c.	Weight of dry mycelium	pH	NH_3–N in 100 c. c.	NH_3–N in 100 c. c.	pH
	Mg.	*Mg.*		*Mg.*	*Mg.*	*Mg.*		*Mg.*	*Mg.*	
Control	8.4	1.76	7.7	8.4	1.76	----	7.7	8.4	1.76	7.7
None	40.28	32.96	8.2	40.70	19.77	94	8.1	----	----	----
Cane sugar	27.54	3.00	4.7	47.17	10.74	173	8.0	----	----	----
Starch	28.33	8.86	5.1	20.17	9.82	643	7.1	----	----	----
Cellulose	----	----	----	[b] 30.32	34.06	96	8.2	[c] 12.88	1.12	6.2

[a] Incubation 8 days; for cellulose flasks, 16 days.
[b] No cellulose decomposed.
[c] 65 mg. of cellulose decomposed.

nitrogen and carbon. The reactions taking place were followed by measuring the residual amino nitrogen formed from decomposition of casein, formation and accumulation of ammonia, amount of growth produced by the organisms, and disappearance of dextrose in the medium, wherever it has been used.

The results indicate that not all organisms attack proteins and amino acids alike. The two fungi *Trichoderma koningi* and *Zygorhynchus molleri* utilized the various amino acids and the protein both as sources of carbon and nitrogen, the amount of growth and ammonia accumulation depending, however, in the absence of available carbohydrates, upon the available carbon in the amino acid molecule. A definite relation was found to exist between the carbon (available) content of the amino acid molecule and the amount of ammonia accumulating. The two bacteria tested, *B. cereus* and *Bact. fluorescens*, behaved differently; the first was unable to attack glycocoll, alanine, and phenylalanine, while glutamic acid and asparagine were acted upon to a limited extent, and casein and other native proteins were decomposed very rapidly. The *Bact. fluorescens* was unable to decompose casein, but acted upon the various amino acids used very readily. By combining the two organisms in casein media, the protein was decomposed very rapidly to ammonia, the *B. cereus* hydrolyzing the casein chiefly to protein derivatives and the *Bact. fluorescens* decomposing the latter to ammonia.

Ammonia accumulation can not be used as an index of the proteolytic activities of microorganisms, when the carbon content of the medium is not considered; an organism may decompose a much larger amount of protein in the presence of an available carbohydrate, but produce a much smaller amount of ammonia.

The Actinomyces was found to be capable of utilizing amino acids and proteins as sources of energy, thus allowing an accumulation of ammonia, even in the presence of dextrose.

Ammonia formation by microorganisms from amino acids depends upon the carbon-nitrogen ratio of the compound, as well as upon the nature of the organism, as influenced by its utilization of energy.

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A BIOMETRIC COMPARISON OF THE UREDINIOSPORES OF CRONARTIUM RIBICOLA AND CRONARTIUM OCCIDENTALE[1]

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INTRODUCTION

It appears to be impracticable to differentiate between *Cronartium ribicola* Fischer and *Cronartium occidentale* Hedgcock, Bethel, and Hunt in the uredinial stage by any ordinary means of visual inspection or low power microscopical examination. Both rusts attack a large number of species of the genus Ribes. Superficially and structurally the uredinia resemble each other very closely; and the urediniospores of the two species differ only slightly—so slightly in fact that the differences escape observation unless special technique is used. The fact that *C. ribicola* is already present in the northwestern United States makes the problem of differentiation something more than a matter of academic interest, on account of the necessity of recognizing advance infections on Ribes.

Hedgcock, Bethel, and Hunt[3] give the size of the urediniospores of *C. occidentale* as 18.5 to 32 by 13.5 to 20μ averaging 24 by 16μ, and state that the wall is 2 to 3μ thick. Colley[4] has described the size of the urediniospores of *C. ribicola* roughly as 19 to 45 by 10 to 20μ. These two range descriptions are not comparable, because the measurements on which the figures are based were not made by similar methods. Granting that they are comparable, however, the range descriptions would not be a sufficiently sound basis for diagnosis, for there is no means of knowing the distribution of the more common spore sizes within the limits of the ranges. Of the many hundreds of spore measurements made on the urediniospores of the two species within the last few years, some have been made on fresh spores and some on dry spores, with and without the use of special mounting media. Obviously these results also are more or less unsatisfactory. The object of this paper is the presentation of an analysis of strictly comparable measurements made on 3,000 urediniospores of each species.

METHODS

SELECTION OF MATERIAL

Herbarium material was selected for the measurement study. Experience had shown clearly that measurements made on fresh spores were not comparable with measurements made on dry spores; and it was obvious that herbarium specimens had one thing at least in common—they were all dry. Furthermore, it was possible to select specimens from the herbarium covering the widest possible range for host, locality, and time of collection. The spores were taken from specimens which appeared to be clean and well preserved, and from sori which appeared to be mature.

The selected specimens were grouped in three series. In each series there were 10 sets of spores—each set consisting of 100 urediniospores of *Cronartium ribicola* and 100 urediniospores of *Cronartium occidentale*. The description of the three series follows:

Field series.—A selection of 50 specimens of each species from collections made in the field; 5 specimens from each species in a set; set numbers 1–10, inclusive.

Block Island series.—A selection of 10 specimens of each species from collections made in experimental plots located on Block Island, off the coast of Rhode Island; one specimen of each species in each set; set numbers 11–20, inclusive.

[1] Received for publication May 16, 1924; issued April, 1925.

[2] The writer wishes to acknowledge his indebtedness to Dr. George G. Hedgcock, Glenn G. Hahn, and Rush P. Marshall of the Office of Investigations in Forest Pathology, and to Miss Minnie W. Taylor, formerly of the office, for their generous assistance during the course of this study.

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Greenhouse series.—A selection of 10 specimens of each species from collections made in the pathological greenhouses at Washington, D. C., one specimen of each species in each set; set numbers 21-30, inclusive.

placed in a small drop of the following medium:[5]

Potassium acetate	10 gms.
Distilled water	500 cc.
Pure glycerine	200 cc.
Ethyl alcohol, 95 per cent	300 cc.
Erythrosin	10 gms.

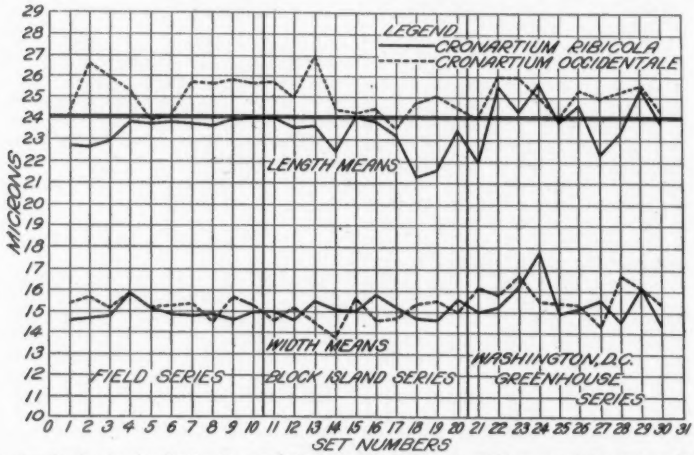

FIG. 1.—Graphs showing the length and width means for 30 sets of urediniospores of *Cronartium ribicola* and *Cronartium occidentale*. Each point on each species line represents the mean of 100 measurements. The series are explained in the text. The set numbers correspond to those in the last columns of Tables I to IV

The collection data are given in full in Tables I to IV.

MOUNTING

The spores were removed from the uredinia as carefully as possible and

They were left in this medium without being covered with a cover glass for several hours. The mount was then finished by adding a drop of glycerine jelly made up as follows:

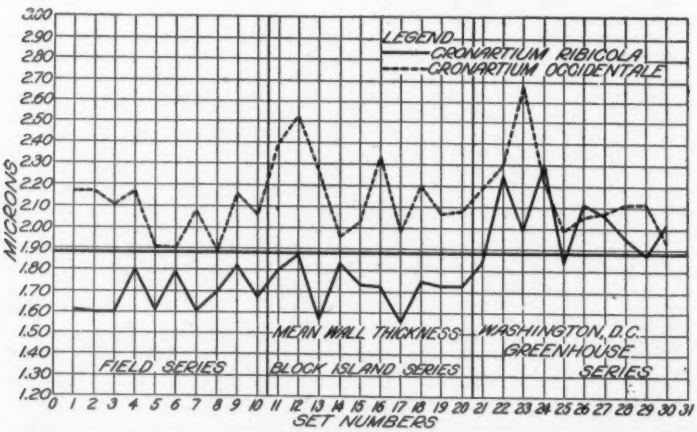

FIG. 2.—Graphs showing the mean wall thickness for 30 sets of urediniospores of *Cronartium ribicola* and *Cronartium occidentale*. Each point on each species line represents the mean of 50 measurements. Series and set numbers as in Figure 1

[5] This medium has been used for years in the Bureau of Plant Industry by Dr C. L. Shear and others. The only modification of their formula is the addition of the erythrosin.

Distilled water	42 cc.
Pure glycerine	50 cc.
Gelatine	7 gms.
Phenol	1 cc.
(Use 1 cc. fresh acid or 1 gm. of crystals.)	
Erythrosin	1 gm. in 9 cc. distilled water

The formula is based on one given by Moreau.[6] The erythrosin should be dissolved as far as possible in the 9 cc. of distilled water and the solution then added to the glycerine gelatine mixture *after* it has been cooked but *before* it has cooled.

MEASURING

All the spores were measured by projection. The apparatus[7] was so arranged that the images of the spores were projected at a magnification of 1,000 diameters on a white field. The images of such spores as fell within a 4-inch circle in this white field were then measured to the nearest millimeter with a standard white-faced millimeter scale. Wall thickness was measured to the nearest half millimeter. The mount was moved across the field of vision systematically by means of a mechanical stage.

In the *Field series* 20 spores were measured from each specimen and the measurements from each 5 specimens grouped at random into sets, as shown in Table I and Table II; but in the *Block Island series* and the *greenhouse series* 100 spores were measured from each specimen. Wall measurements were made on 50 of each 100 spores measured. For each series, therefore, there were 1,000 length measurements, 1,000 width measurements, and 500 wall measurements.

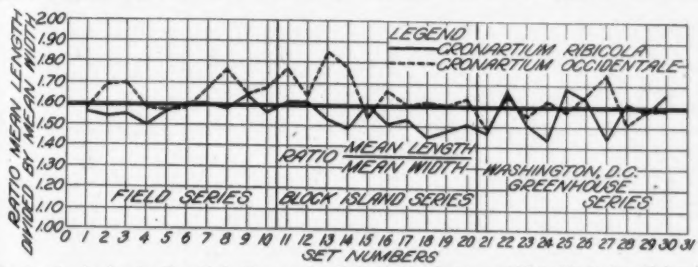

FIG. 3.—Graphs showing the ratio for the mean length divided by the mean width for 30 sets of urediniospores of *Cronartium ribicola* and *Cronartium occidentale*. Series and set numbers as in Figure 1

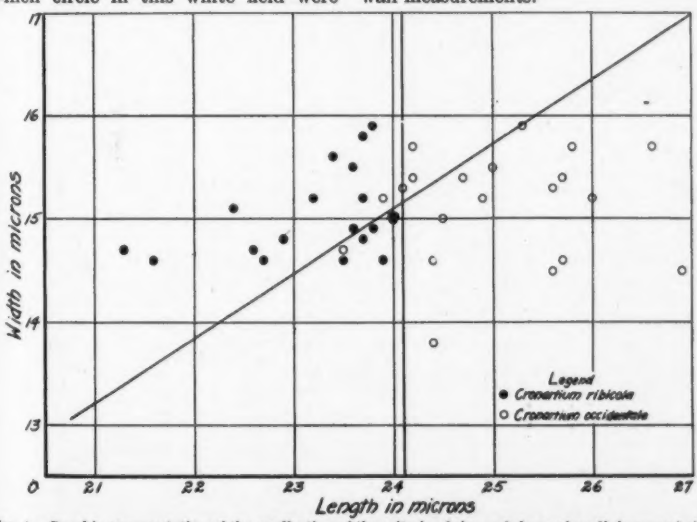

FIG. 4.—Graphic representation of the application of the criteria of size and shape of urediniospores to the biometric diagnosis of *Cronartium ribicola* and *Cronartium occidentale*. Each plotted point represents the length and width mean of 100 measurements. The data are taken from the figures given in Tables I–III for sets 1–20. Further explanation in the text

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ANALYSIS OF THE MEASUREMENT RESULTS

The measurements were tallied and analyzed by ordinary statistical methods. The means for the length and width and wall thickness and the ratio of mean length divided by mean width for each of the 30 sets of spores are given in Tables I to IV. A summary of the measurement data is given in Table V.

The data in Tables I to IV are presented graphically in Figures 1, 2, and 3. Each point on the graphs in Figure 1 represents the mean of the measurements of 100 spores; each point on the graphs in Figure 2 represents the mean of the measurements of the walls of 50 spores; and each point on the graphs in Figure 3 represents the quotient obtained by dividing the mean of the length measurements of 100 spores by the mean of the width measurements of the same spores. The points are connected by the solid line for *Cronartium ribicola* and the broken line for *Cronartium occidentale* in order to facilitate reading.

TABLE I.—*Field series; means for each 100 urediniospores measured; Cronartium ribicola*

Forest pathology No.	Host	Locality	Date collected	Mean length	Mean width	Mean wall thickness	Ratio mean length / mean width	Set No.
2607	Ribes vulgare	Font Hill, Ont.	June 6, 1915					
23735	do	North Conway, N. H.	July 4, 1917					
34070	do	Red Bank, N. J.	Aug. 13, 1919	22.7	14.6	1.61	1.56	1
23870	do	Rochester, N. Y.	Aug. 2, 1917					
22216	do	Chalmers, Mass.	Aug. 11, 1916					
34430	do	Pomroy, Minn.	July 26, 1919					
23105	do	Little Compton, R. I.	Aug. 17, 1916					
23205	do	Acton Corner, Me.	Aug. 30, 1916	22.6	14.7	1.60	1.54	2
23254	Ribes reclinatum	Bar Harbor, Me.	Oct. 2, 1916					
22443	do	Newport, R. I.	Sept. 5, 1916					
2592	do	Lyndonville, Vt.	Sept. 12, 1914					
34260	do	Shafer, Minn.	Sept. 29, 1919					
23888	Ribes nigrum	Rochester, N. Y.	Aug. 18, 1917	22.9	14.8	1.60	1.55	3
23012	do	Warwick, R. I.	July 24, 1916					
23846	do	North Whately, Mass.	July 26, 1917					
34442	do	Cannon City, Minn.	July 30, 1919					
23902	do	Tupper Lake, N. Y.	Aug. 22, 1917					
24021	do	Red, Bank, N. J.	Aug. 21, 1918	23.8	15.9	1.80	1.50	4
20623	do	Font Hill, Ont.	Aug. 13, 1915					
25337	do	Temple, N. H.	June 10, 1919					
23218	do	Limerick, Me.	Sept. 4, 1916					
34036	do	Amery, Wis.	July 11, 1919					
23020	Ribes hirtellum	Lyman, Me.	July 25, 1916	23.7	15.2	1.61	1.56	5
23734	do	Bath, N. H.	July 2, 1917					
34435	Ribes oxyacanthoides	Pomroy, Minn.	July 28, 1919					
29632	do	Rice Lake, Wis.	Sept. 18, 1918					
24043	Ribes cynosbati	West Greenwich, R. I.	Oct. 15, 1918					
24032	do	Bartlett, N. H.	June 13, 1918	23.8	14.9	1.79	1.60	6
20695	do	Norfolk, Conn.	June 13, 1916					
2606	do	Font Hill, Ont.	June 26, 1915					
23868	do	Stephentown, N. Y.	Aug. 4, 1917					
34446	do	Sec. 26, R. 21, Minn.	Aug. 1, 1919					
34103	do	Clear Lake, Wis.	July 23, 1919	23.7	14.8	1.61	1.60	7
25334	do	Bethel, Vt.	July 6, 1919					
22137	Ribes americanum	Central Village, Mass.	July 6, 1916					
24045	do	Font Hill, Ont.	June 22, 1917					
23764	do	St. Johnsbury, Vt.	July 20, 1917					
34235	do	Munch Township, Minn.	Sept. 21, 1919	23.6	14.9	1.70	1.58	8
34537	do	Downing, Wis.	Aug. 26, 1919					
22165	Ribes glandulosum	Norfolk, Conn.	July 3, 1916					
23859	do	Bartlett, N. H.	July 28, 1917					
28232	do	Brunswick, Me.	May 22, 1918					
23877	do	North Thetford, Vt.	Aug. 7, 1917					
24321	do	Amery, Wis.	Aug. 11, 1916	23.9	14.6	1.82	1.64	9
24019	Ribes triste	Tuckerman Ravine, N. H.	Aug. 3, 1918					
23365	do	Rush Lake, Minn.	June 14, 1918					
34095	Ribes odoratum	Petersham, Mass.	July 30, 1919					
23125	do	Kittery, Me.	Aug. 18, 1916	24.0	15.0	1.68	1.56	10
23834	do	Keeseville, N. Y.	July 24, 1917					
23798	do	Orford, N. H.	July 11, 1917					

TABLE II.—*Field series; means for each 100 urediniospores measured; Cronartium occidentale*

Forest pathology No.	Host	Locality	Date collected	Mean length	Mean width	Mean wall thickness	Ratio mean length / mean width	Set No.
27029	Ribes aureum	Farmington, N. Mex.	Sept. 9, 1918					
27033	do	Aztec, N. Mex.	Sept. 10, 1918					
27151	do	Clayton, N. Mex.	Sept. 26, 1919	24.2	15.4	2.17	1.57	1
27154	do	Farmington, N. Mex.	Aug. 2, 1919					
29087	do	Miller Valley, Ariz.	Oct. 6, 1917					
28218	do	Monrovia, Calif.	May —, 1919					
28275	do	do	May 13, 1919					
29518	do	Morgan, Utah	Aug. 24, 1920	26.6	15.7	2.17	1.69	2
29519	do	Iron Co., Utah	July 30, 1920					
26475	Ribes odoratum	Prescott, Ariz.	Oct. 2, 1917					
29515	Ribes aureum	Fillmore, Utah	Aug. 7, 1920					
29516	do	Hygrum, Utah	Aug. 22, 1920					
29517	do	Scipio, Utah	Aug. 10, 1920	26.0	15.2	2.11	1.70	3
36221	do	Iron Co., Utah	July 30, 1920					
36222	do	Beaver, Utah	Aug. 3, 1920					
27100	do	Hayden, Colo.	Oct. 18, 1918					
26159	do	Meeker, Colo.	Oct. 2, 1917					
27046	do	Trimble Hot Springs, Colo.	Sept. 14, 1918	25.3	15.9	2.17	1.58	4
27047	do	Hermosa, Colo.	Sept. 15, 1918					
27049	do	Durango, Colo.	Sept. 15, 1918					
32707	Grossularia velutina	Mono Lake, Calif.	Aug. 20, 1919					
34076	Ribes aureum	Bridgeport, Calif.	Aug. 2, 1919					
36621	Ribes gracillimum	do	Aug. 20, 1919	23.9	15.2	1.91	1.57	5
34019	Grossularia inermis	Denver, Colo.	July 1, 1919					
34020	do	do	July 1, 1919					
29525	do	Monticello, Utah	Aug. 15, 1918					
29654	Grossularia reclinata	Mancos, Colo.	Sept. 12, 1918					
22644	do	Prescott, Ariz.	Oct. 27, 1917	24.1	15.3	1.90	1.58	6
29392	do	Mancos, Colo.	July 31, 1918					
26745	Ribes aureum	Pagoro Springs, Colo.	Sept. —, 1917					
22648	Ribes odoratum	Prescott, Ariz.	Oct. 29, 1917					
22635	do	do	Oct. 27, 1917					
29463	do	Mancos, Colo.	Aug. 9, 1918	25.7	15.4	2.08	1.67	7
22634	Ribes aureum	Prescott, Ariz.	Oct. 27, 1917					
36112	do	Monrovia, Calif.	June 15, 1920					
28294	Ribes gracillimum	Monrovia, Calif.	June 25, 1919					
28277	Ribes aureum	do	May 18, 1919					
28276	do	do	May 17, 1919	25.6	14.5	1.80	1.76	8
23396	do	do	May 22, 1919					
25054	Ribes odoratum	do	Oct. 27, 1919					
26485	Ribes aureum	Naturita, Colo.	Aug. 18, 1917					
26499	do	Denver, Colo.	Sept. 25, 1917					
27101	do	Craig, Colo.	Oct. 18, 1918	25.8	15.7	2.16	1.65	9
27039	do	La Plata, Colo.	Sept. 11, 1918					
36059	do	Monrovia, Calif.	Feb. 25, 1919					
2859	do	Boulder, Colo.	July —, 1914					
24648	do	Ute Reservation, Colo.	July —, 1897					
22618	do	Bayfield, Colo.	Aug. 26, 1917	25.6	15.3	2.06	1.68	10
24420	do	do	Sept. 15, 1917					
36089	do	Monrovia, Calif.	June 14, 1920					

TABLE III.—*Block Island series; means for each 100 urediniospores measured; Cronartium ribicola and Cronartium occidentale*

CRONARTIUM RIBICOLA

Forest pathology No.	Host	Locality	Date collected	Mean length	Mean width	Mean wall thickness	Ratio mean length / mean width	Set No.
34555	Ribes odoratum.	Block Island, R. I.	June 22, 1920	24.0	15.0	1.80	1.61	11
34556	do	do	June 22, 1920	23.5	14.6	1.87	1.61	12
34579	do	do	July 20, 1920	23.6	15.5	1.57	1.53	13
34622	do	do	Sept. 8, 1920	22.4	15.1	1.83	1.49	14
34878	do	do	Sept. 30, 1920	24.0	15.0	1.74	1.60	15
34881	do	do	Sept. 30, 1920	23.8	15.8	1.73	1.51	16
34558	Ribes nigrum (Naples).	do	June 22, 1920	23.2	15.2	1.56	1.53	17
34619	do	do	Sept. 8, 1920	21.3	14.7	1.75	1.45	18
34888	Ribes americanum.	do	Sept. 30, 1920	21.6	14.6	1.73	1.48	19
34880	Ribes prostratum.	do	Sept. 30, 1920	23.4	15.6	1.73	1.51	20

CRONARTIUM OCCIDENTALE

34568	Ribes odoratum.	Block Island, R. I.	June 23, 1920	25.7	14.6	2.39	1.77	11
34574	do	do	June 30, 1920	24.9	15.2	2.52	1.64	12
34630	do	do	Sept. 9, 1920	26.9	14.5	2.26	1.85	13
34634	do	do	Sept. 9, 1920	24.4	13.8	1.96	1.77	14
34846	do	do	Sept. 9, 1920	24.2	15.7	2.03	1.54	15
34877	do	do	Oct. 1, 1920	24.4	14.6	2.34	1.67	16
34627	Ribes nigrum (Naples).	do	Sept. 9, 1920	23.5	14.7	1.99	1.60	17
34871	do	do	Oct. 1, 1920	24.7	15.4	2.20	1.61	18
34647	Ribes americanum.	do	Sept. 9, 1920	25.0	15.5	2.07	1.60	19
34633	Ribes prostratum.	do	Sept. 9, 1920	24.5	15.0	2.08	1.63	20

TABLE IV.—*Washington, D. C., greenhouse series; means for each 100 urediniospores measured; Cronartium ribicola and Cronartium occidentale*

CRONARTIUM RIBICOLA

Forest pathology No.	Host	Locality	Date collected	Mean length	Mean width	Mean wall thickness	Ratio mean length / mean width	Set No.
34684	Ribes aureum	Washington, D. C.	July 15, 1920	22.0	15.0	1.84	1.47	21
34697	do	do	Aug. 2, 1920	25.5	15.2	2.24	1.67	22
34722	do	do	Aug. 25, 1920	24.3	16.1	1.99	1.51	23
34826	do	do	Oct. 8, 1920	25.6	17.7	2.29	1.44	24
36466	do	do	July 21, 1920	23.8	14.9	1.84	1.68	25
34712	do	do	Aug. 11, 1920	24.6	15.1	2.11	1.63	26
34740	do	do	Aug. 4, 1920	22.3	15.5	2.06	1.44	27
36125	do	do	July 8, 1920	23.3	14.5	1.95	1.61	28
36465	do	do	Aug. 9, 1920	25.4	16.1	1.87	1.57	29
36470	do	do	Aug. 9, 1920	23.7	14.3	2.01	1.66	30

CRONARTIUM OCCIDENTALE

36104	Ribes aureum	Washington, D. C.	July 1, 1920	24.0	16.1	2.19	1.49	21
36150	do	do	July 2, 1920	26.0	15.8	2.29	1.65	22
36233	do	do	Aug. 17, 1920	25.9	16.7	2.66	1.55	23
36406	do	do	May 22, 1920	25.0	15.5	2.22	1.62	24
36439	do	do	Aug. 2, 1920	24.0	15.4	1.99	1.57	25
36446	do	do	Aug. 9, 1920	25.3	15.3	2.05	1.65	26
36078	do	do	July 8, 1920	24.9	14.3	2.07	1.75	27
36131	do	do	July 14, 1920	25.2	16.7	2.11	1.51	28
36404	do	do	June 22, 1920	25.5	16.1	2.11	1.58	29
36429	do	do	June 9, 1920	24.2	15.3	1.93	1.58	30

TABLE V.—*Summary of measurement data; urediniospores of Cronartium ribicola and Cronartium occidentale*

Species	Length [a]			Width			Mean [b] wall thickness	Ratio mean length / mean width
	Mean length	Standard deviation	Coefficient of variability	Mean width	Standard deviation	Coefficient of variability		
Field series:								
Cronartium ribicola	23.5	2.6	11.1	15.0	1.8	12.1	1.70	1.57
Cronartium occidentale	25.2	3.5	13.7	15.3	1.8	11.4	2.05	1.64
Block Island series:								
Cronartium ribicola	23.1	2.6	11.3	15.1	1.6	10.8	1.73	1.53
Cronartium occidentale	24.9	2.9	11.8	14.9	1.7	11.5	2.18	1.67
Washington, D. C., greenhouse series:								
Cronartium ribicola	24.1	3.4	13.9	15.4	2.0	12.9	2.02	1.57
Cronartium occidentale	25.0	3.1	12.3	15.7	1.9	12.0	2.16	1.60

[a] The length and width means are based on 1,000 measurements for each species in each series.
[b] The mean wall thickness is based on 500 measurements for each species in each series.

DISCUSSION

Each of the three series of measurements serves a different purpose; for the mean measurements of the sets in the *Field series* evidently are better species indices than those in the two other sets, being based on a broader sampling system; and the means in the *Block Island series* show the difference in size of the urediniospores of the two species on a few Ribes hosts growing in the same locality, as well as the practical utility of 100 spore means for diagnostic purposes; whereas the means in the *Greenhouse series* represent the variation in the urediniospores of the two species on the same host (*Ribes aureum*) grown under experimental conditions in the greenhouse.

It is evident from the graphs of the length means and wall thicknesses that the two species are distinct, as far as these two factors are concerned, in the *Field series* and *Block Island series*, but that they are not separable on the same bases in the *Greenhouse series*. The respective value of the measurements for diagnostic purposes appears to be in the order of length mean, mean wall thickness, and ratio of mean length divided by mean width. The width mean has no diagnostic value. The results indicate that measurements of the urediniospores from hosts growing in the greenhouse should be used with extreme caution, if at all, in any attempt to diagnose field collections of either species. The data obtained in the *Field series* and the *Block Island series*, however, appear to warrant the conclusion that field collections can be separated in most cases on the basis of spore size, spore shape, and wall thickness. The following discussion of diagnostic division points is accordingly confined to the data from sets 1–20 inclusive.

The horizontal line drawn at 24.1μ, in Figure 1, separates 37 out of the 40 length means correctly, all 20 means for *C. ribicola* being below the line and 17 means for *C. occidentale* being above the line. One mean for *C. occidentale* falls on the line.

The horizontal line drawn at 1.88μ, in Figure 2, separates all 40 of the mean wall thicknesses correctly, the 20 for *C. ribicola* being below the line and the 20 for *C. occidentale* being above the line.

The horizontal line drawn at 1.59, in Figure 3, separates 29 out of 40 of the ratios of mean length divided by mean width correctly, 14 out of 20 for *C. ribicola* being below the line and 15 out of 20 for *C. occidentale* being above the line.

In other words the diagnostic division points,

24.1 for the length mean,
1.88 for the mean wall thickness, and
1.59 for the ratio of mean length divided by mean width,

would have correctly identified 92.5 per cent, 100 per cent, and 72.5 per cent, respectively, of the sets measured.

Examination of the graphs shows that the measurements for *C. occidentale* in set 5 are abnormal, that is, on the wrong side of the diagnostic division points for both length mean and ratio; that the figures are low for the length mean of the same species in set 6, low for the wall mean in sets 5 and 6, and below the line for the ratio in set 6. However, there is not a single case where the figures for any one set for either species fall on the wrong side of the line for all three criteria, and only one case, the one in set 5 men-

tioned above, where the figures fall on the wrong side for two criteria. On account of the greater irregularity in the ratio figures, a variation above or below the line is of less importance than a similar variation in length or wall thickness mean. If the two latter criteria only are considered there are but 2 means out of 40 which are definitely out of line—the length means for *C. occidentale* in sets 5 and 17—which makes the biometric diagnosis correct in 95 per cent of the trails.

The relation of the means of sets 1 to 20 to the diagnostic division points for the length mean and for the ratio of mean length divided by mean width is shown again in Figure 4, a type of figure which appears to be particularly satisfactory for illustrating the application of the criteria of size and shape to biometric diagnosis. Each of the plotted points represents both the mean length and mean width of 100 measurements. The means for *C. ribicola* are shown as dots and the means for *C. occidentale* are shown as circles. Set numbers have been omitted purposely, but the position of the means for any set can be found by reference to the mean values given in Tables I to III. For example, the means for set 1 are indicated by the dot and circle at 22.7 and 14.6, and at 24.2 and 15.4, respectively.

The diagnostic division point for the length mean, represented by the horizontal line drawn at 24.1 in Figure 1, is here represented as a vertical line at 24.1. The diagonal line, drawn to satisfy the equation $y=\frac{x}{1.59}$, represents the diagnostic division point for mean length divided by mean width. The figure illustrates clearly the "scatter" or shotgun pattern of the mean sizes for each of the 100's measured, the fact that length is the factor governing the distribution of the points to the left or right of the 24.1 line, and the effect of variation in size on the position of the plotted points with respect to the diagonal.

The difference between the 1,000 spore length means of the two species (see Table V) for the *Field series* is 1.76 ± 0.092, for the *Block Island series* 1.82 ± 0.072, and for the *Greenhouse series* 0.81 ± 0.099. No attempt will be made at present to establish the significance of these differences with respect to their probable errors, since it seems preferable to leave any such discussion as well as a more detailed description of the mounting measuring and analysis methods to a later paper.

The ranges for the spore sizes of the two species are given in Table VI. It is obviously impossible to separate the species on the basis of the range of the spore sizes. Ranges obtained by subtracting and adding the standard deviation to the mean for each dimension—which might be called "standard ranges"—are, however, more significant. These ranges in the three series are given in Table VII. In spite of the fact that the standard ranges are much more accurate indices of the spore sizes than the complete ranges, they do not serve to bring out the differences between the species as clearly as the tabular or graphic representation of the means.

It must be admitted after examination of the graphs in Figures 1 and 2 that *C. ribicola* and not *C. occidentale* is the species which appears to be "abnormal" under greenhouse conditions. No adequate explanation of the "abnormality" is possible at present.

In the *Field series* the measurements of 5 lots of 20 spores each were grouped together, as has been previously stated,

TABLE VI.—*Ranges for length and width of the urediniospores of Cronartium ribicola and Cronartium occidentale*

Series	C. ribicola		C. occidentale	
	Length	Width	Length	Width
Field	16–34	10–22	16–37	10–21
Block Island	13–32	10–21	18–37	10–22
Greenhouse	15–40	10–24	16–39	10–23

TABLE VII.—*Standard ranges for the length and width of urediniospores of Cronartium ribicola and Cronartium occidentale*

Series	C. ribicola		C. occidentale	
	Length	Width	Length	Width
Field	20.9–26.1	13.2–16.8	21.7–28.7	13.5–17.1
Block Island	20.5–25.7	13.5–16.7	22.0–27.8	13.2–16.6
Greenhouse	20.7–27.5	13.4–17.4	21.6–28.4	13.5–17.3

and the means for length and width illustrated in Figure 1 were based on the resultant 100's. This procedure undoubtedly resulted in a set of means showing less variation than would be expected if the means had been based on the measurements of 100 spores from single specimens, in which case there would have been more danger of crossing the dead line 24.1. The points on the graph for the length means of sets 1–10 in the *Field series* are, therefore, not exactly comparable with the points for sets 11–20 in the *Block Island series*. As a matter of fact, the *Block Island series* is the only one of the three which illustrates the way in which biometric diagnosis methods based on measurements of 100 spores might work out when applied to unknowns.

The data indicate that 200 measurements would have been better than 100 as a basis for determining the means; and, in cases where the species are close together as these rusts are, it would seem wiser to use the higher number. Means based on 200 measurements would probably have shown less tendency to cross the diagnostic division point than the means based on 100 measurements.

It would be preferable also in diagnosing unknown specimens to take spores from as many different leaves or sori as possible for each specimen studied; in other words, to sample the specimen with the aim of getting the best possible representative lot of spores for the mount. Unfortunately it was not practicable to follow this course in all the cases reported in this paper.

The following examples indicate the futility of attempts to use measurements of fresh spores as size standards, particularly in the case of *C. ribicola*, unless, of course, they are to be compared with other measurements made on fresh spores. The mean measurements for 137 fresh urediniospores of *C. ribicola* from *Ribes gracillimum*, mounted in water, were found to be 28.7 by 18.4, with a standard range of approximately 25 to 33 by 16 to 23μ. The walls of this set were not measured. The means of 50 fresh urediniospores, mounted in water, from a specimen collected on *Ribes aureum* growing in the greenhouse were 33.2 by 21.5μ, with a standard range of approximately 30 to 37 by 20 to 24μ. The mean wall thickness was 0.91μ. These means and standard ranges are far out of line with the figures given in the tables for *C. ribicola*. On the other hand the means for 330 urediniospores from herbarium specimens of *C. ribicola* collected in the field on various hosts were 22.3 by 15.4μ, with a standard range of 18 to 27 by 13 to 18μ. The mean wall thickness was approximately 2.00μ. These figures agree fairly well with those for sets 1, 2, and 3 of *C. ribicola* in Table I, except for the wall mean; yet the results are not really comparable, except in a very general way, because the measurements were made some years ago by methods which were not comparable with those used by the writer.

On the basis of the data presented, it would be going too far to expect biometric diagnosis methods to yield 100 per cent correct results; but they are the only methods applicable in cases where one is dealing with herbarium material, and where inoculation experiments are impossible. The diagnostic division points given in this paper can not be expected to hold good unless the mounting methods are rigidly followed. The measuring must be done with great care—as all real measuring should be done—and the measurements should be analyzed by statistical methods. Under such conditions the investigator can separate the uredinial stages of *C. ribicola* and *C. occidentale* in most cases with comparative ease.

In a later paper a biometric comparison of the aeciospores of the two species will be presented.

SUMMARY

The above analysis of measurements on 3,000 urediniospores of *Cronartium ribicola* and *Cronartium occidentale* indicates that the two species may be separated in the uredinial stage with practical certainty on the basis of spore size, shape, and wall thickness.

The most important criteria for biometric diagnosis are the length mean, the mean wall thickness, and the ratio of mean length divided by mean width.

The diagnostic division point for the length mean is 24.1μ, for the mean wall thickness 1.88μ, and for the ratio of mean length to mean width 1.59.

In the cases of collections from the field and from experimental plots these three diagnostic division points proved good in 92.5 per cent, 100 per cent, and 72.5 per cent, respectively, of the trials.

The dimensions of urediniospores produced under greenhouse experimental conditions do not appear useful for distinguishing these two species.

The data presented in the paper are applicable only when the conditions of mounting, measuring, and analysis are strictly comparable.